Society for
Reproduction
and Fertility

ANNUAL CONFERENCE 2009

12 – 14 July 2009

St Catherine's College, Oxford

PROGRAMME AND ABSTRACT BOOK

www.srf-reproduction.org

Contents

Welcome

Welcome to the Society for Reproduction and Fertility's (SRF) Annual Conference 2009 at St Catherine's College, Oxford.

This meeting will be attended by members of the scientific community that include programme leaders, postdoctoral fellows and PhD students working within research and academic institutions across the UK, Europe, USA and Australasia.

For the 2009 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 the recipient of the Society's Marshall Medal is Professor Twink Allen 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 Prize) will also be presented at the Conference Dinner.

The conference programme includes FREE COMMUNICATIONS in the general areas of *Reprogramming*, *Female Reproduction, Ovary and Oocytes*, MULTIPLE SYMPOSIA (*Lifestyles and Reproduction*, *Communication in Pregnancy* and *Non-transcriptional Modifications)* and PLENARY LECTURES, the Distinguished Scientist (DM Stocco), SSR New Investigator (HH Yao) and SRF New Investigator (KJ Sales). The ever popular SRF STUDENT PRIZE and the POST DOCTORAL PRIZE sessions 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".

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 2009 set in the beautiful city of Oxford. I hope you find it beneficial and informative, and that you enjoy your time at SRF 2009.

Dr Robert Abayasekara
SRF Programme Secretary

General Information

Oxford and Venue

Oxford, *The City of Dreaming Spires*, is famous for its University and place in history. It has been a home to royalty and scholars for over 800 years. With its ancient University and growing modern community, the city is now an exciting cosmopolitan town.

This historic and modern city has plenty to offer visitors to the area. From visiting one of the many historic buildings, colleges or museums to dining out, going to the theatre or shopping, Oxford has something for everyone.

Within the city stands the youngest undergraduate College, St Catherine's. It is a distinguished example of the very best of architecture in the tradition of European Modernism. The College, designed by the Danish architect, Arne Jacobsen, described by Pevsner as "a perfect piece of architecture", is one of only a very few pieces of post-1945 architecture to be Grade 1 listed. Jacobsen's buildings are complemented by three new residential blocks, built by Stephen Hodder MBE in the early 1990s and, more recently, in 2004.

St Catherine's offers the benefits of modern accommodation, excellent catering and up-to-date meeting and technical facilities, whilst being only a short walk from the cloisters and quads of traditional Oxford. It lies in extensive gardens and wooded areas just to the north of Magdalen Bridge in the Holywell Great Meadow, between two branches of the River Cherwell.

For further information about the venue please visit http://www.stcatz.ox.ac.uk/

Car Parking

There is very limited car parking at the College and it is therefore recommended that guests use public transport to travel to the conference.

Trains run at least once an hour between Oxford and London, and twice an hour during peak times. Oxford is also on the main cross-country routes. For details of times and fares visit http://www.rail.co.uk. Taxis are recommended from the station.

Two companies run frequent buses, 24 hours a day, between London (Victoria Bus Station) and Oxford. These are the Oxford Bus Company telephone 01865 785 400, and Oxford Tube telephone 01865 772 250. There are also direct and regular services to Oxford from Heathrow and Gatwick airports operated by the Oxford Bus Company, tel: 01865 785 400.

Coach services from other parts of the country are available with National Express.

Accommodation and Breakfast

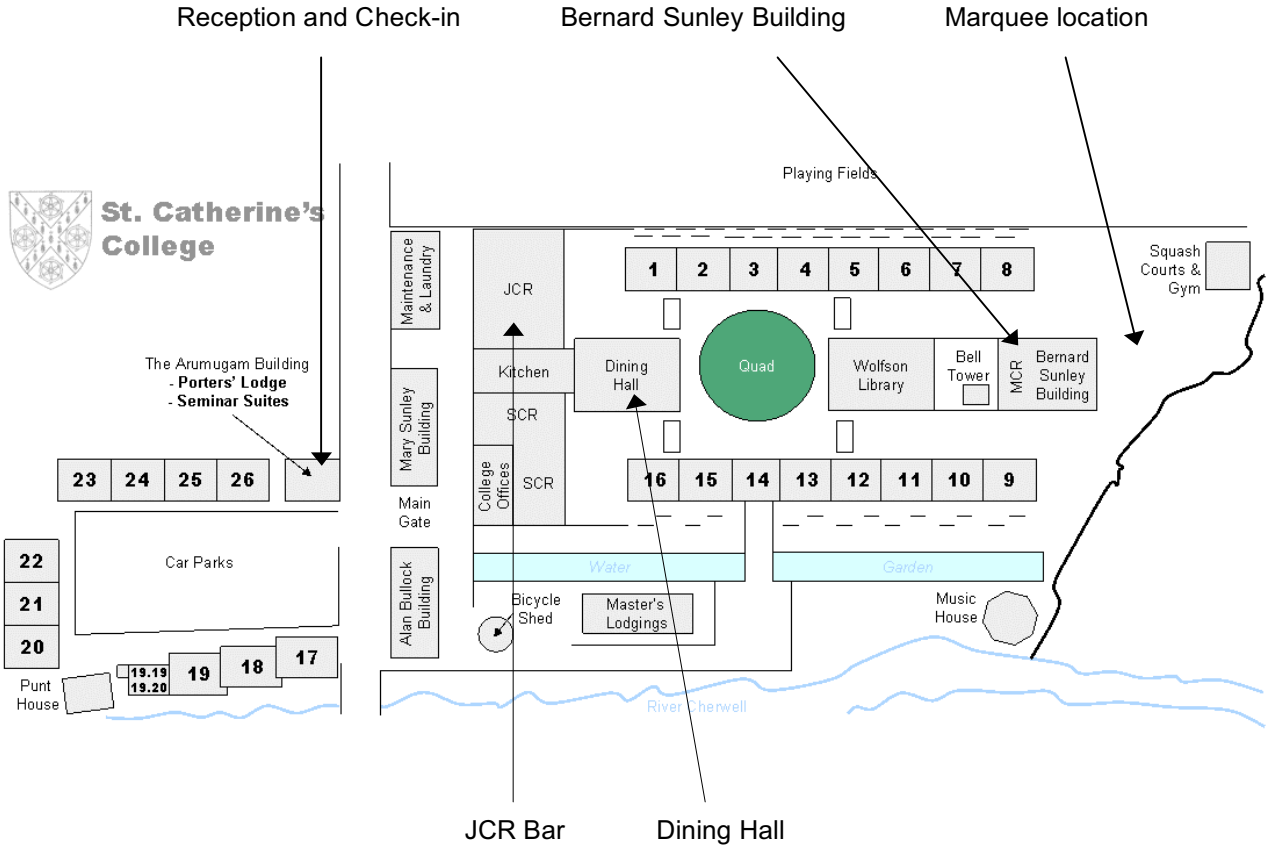
For delegates who have booked a residential package, all accommodation is housed within the College Campus. Guests may check in at any time from 1400 and are required to check out at 1000. It will be necessary to charge for an additional night if guests are unable to leave before this time. There is a luggage room available at the Reception should any guests require this service.

Breakfast is included in the residential packages and is served from 0800 in the main Dining Hall.

Campus Map

St Catherine's College
Manor Road
Oxford
OX1 3UJ

Telephone: +44 (0)1865 271700
Website: http://www.stcatz.ox.ac.uk



Conference Information

Registration

The registration desk will be situated in the Porters' Lodge where you will also be able to check-in for your accommodation. A registration desk will also be located in the foyer of the Bernard Sunley Building should any delegates need assistance throughout the conference.

Opening times:
Sunday 12th July: 1130 – 1800
Monday 13th July: 0800 – 1800
Tuesday 14th July: 0900 – 1330

Delegates will be able to collect their delegate packs from the registration desk at the above times. Packs will include a Programme and Abstract Book, certificate of attendance, badge and holder as well as any social tickets ordered.

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 Bernard Sunley Building. Please refer to the full programme for details of exact session/room locations.

Catering and refreshments will be served throughout the conference. All tea breaks will be served in the Marquee. Please refer to the Social Functions for details of their location.

Poster Sessions

Posters will be on display in the Marquee throughout the conference.

Poster viewing sessions:
Sunday 12th July, 1800 – 1930 (Drinks reception)
Monday 13th July, 1530 – 1700 (Tea break)

Awards

The Awards Presentation will take place in the Dining Hall during the Conference Dinner on Monday 13th July. During the dinner, Professor Twink Allen 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 13th July from 1230 to 1300 in the Bernard Sunley Lecture Theatre.

Social Programme

Sunday 12th July

Dress code: Casual

Drinks Reception
The first night of the conference begins with a Drinks Reception in the Marquee from 1800 to 1930.

All delegates and guests are invited to this reception to enjoy a glass of wine together whilst viewing the poster presentations.

SRF Quiz
The SRF Quiz will follow the Drinks Reception in the Marquee. This now infamous Quiz is not to be missed for a real fun and entertaining evening.

A buffet supper will be served from 1930 to 2030. Delegates and guests will then move to the JCR for later drinks.

All delegates and guests are welcome to attend.

Monday 13th July

Dress code: Smart casual

Pre-dinner Drinks
Pre-dinner Drinks will be served in the JCR 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 Hall at 2000. Guests will then move to the JCR for later drinks and entertainment.

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

During the evening, the SRF Awards Presentation will take place, presenting Professor WR "Twink" Allen with the Marshall Medal and recognising all other SRF Prize winners.

Following the meal, guests will move to the JCR where a local DJ will provide the music that will keep delegates dancing late into the night.

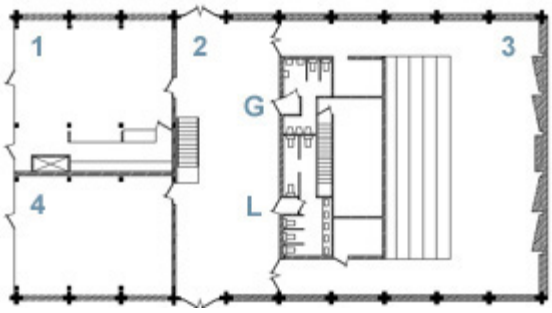
A cash bar will be available in the JCR.

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

Bernard Sunley Building

The Bernard Sunley Building will host all the conference sessions. Please see details below of the floor plan for this building and the rooms that will be used for the SRF Conference.

Ground Floor



Key to ground floor

1 Not in use

2 Foyer (with Registration Desk)

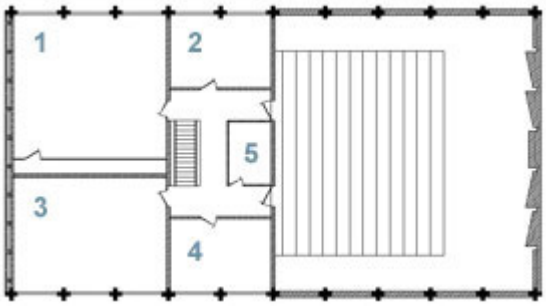
3 Bernard Sunley Lecture Theatre

4 Room A (Parallel Sessions)

L Ladies' toilet

G Gentlemen's toilet

First Floor



Key to first floor

1 Not in use

2 Law Library (Speaker Room)

3 Room C (Parallel Sessions)

4 Room B (Organiser's Office)

5 Projection Room

Programme - Sunday 12th July

TIME	SESSION DETAILS
1200 - 1300	**SRF Council Meeting (SRF Council Members only)**
1400 - 1600	**Symposium 1: Lifestyles and Reproduction** (Bernard Sunley Lecture Theatre) Age and reproductive fitness: evolutionary theory and biogerontological practice – D Gems Physiological and man-made influences on fertility in thoroughbred horses – WR Allen Impact of obesity on maternal reproductive fitness – FC Denison **Chair: MR Luck**
1600 - 1630	**Tea/Coffee Break (Marquee)**
1630 - 1800	**Post Doc Scientist Award** (Bernard Sunley Lecture Theatre) **O1** Androstenedione does not overcome transforming growth factor-β1 inhibition of FSH receptor mRNA expression in cultured bovine granulosa cells – XF Zheng **O2** Dynamic changes in the expression and functionality of BMP signalling accompanies the transition from germ cell proliferation to differentiation in the human fetal ovary – AJ Childs **O3** Abnormal development of mouse preantral follicles in vitro – SA Stubbs **O4** Prostaglandin synthesis and signalling in the porcine endometrium and conceptus – A Waclawik **Chair: G FitzHarris**
1800 - 1930	**Welcome Drinks Reception and Poster Viewing (Marquee)**
1930 - 2300	**SRF Quiz with Buffet (Marquee)**

Programme – Monday 13th July

TIME	SESSION DETAILS		
0830 - 1030	**Symposium 2: Communication in Pregnancy** (Bernard Sunley Lecture Theatre) Intercellular communication in oogenesis: implications for pregnancy – GM Kidder How does the maternal immune system respond to trophoblast? – A Moffett Trophoblast-maternal interactions: what can we learn from the mare? – AM de Mestre **Chair: F Houghton**		
1030 - 1100	**Tea/Coffee Break (Marquee)**		
1100 - 1230	**Oral Session 1: Reprogamming** (Bernard Sunley Lecture Theatre) **O5** Role of P2X receptors in the activation of anion secretion in the bovine oviduct epithelium – A Fitzgerald **O6** Human Cumulus Cell Gene Expression as a Biomarker of pregnancy outcome following single embryo transfer – KM Gebhardt **O7** Parthenogenetic activation of ovine oocytes vitrified at germinal vesicle stage – AR Moawad **O8** Comparison of the behavioural responses of Yellow Coat Colour Avy/a Mice to Brown Coat Colour (Pseudoagouti) Avy/a Sibling Mice – CS Rosenfeld **O9** Simplifying Sheep Androgenetic Embryo Production – F Zacchini **O10** Induced pluripotent stem cells derived from pigs – RM Roberts **Chairs: P Loi and HH Yao**	**Oral Session 2: Female Reproduction** (Room A) **O11** Effect of FSH and 17β-oestradiol on *in vitro* levels of PGE_2 and COX-2 in explants of cervical tissue from ewes in the follicular phase – L Falchi **O12** Foetal long-term implantation with a GnRH agonist reduces the number of primordial follicles in 6 week-old female calves – JH Hernandez-Medrano **O13** Effect of n-3 polyunsaturated fatty acid supplementation on prostaglandins produced by uterine endometrial cells isolated from cyclic ewes – Z Cheng **O14** Unbalanced expression of a panel of relevant imprinted and non-imprinted genes regulating placental growth and angiogenesis in sheep foetuses produced by Assisted Reproductive Technologies (ART) – P Toschi **O15** Contractions induced by prostaglandin (PG) E_2 and $PGF_{2\alpha}$ are superseded by thromboxane in isolated human myometrium at term pregnancy – DP Fischer **O16** Regulation of angiogenesis and lymphangiogenesis in human endometrium via PGF2alpha-FP initiation of adrenomedullin signalling – S Battersby **Chairs: APF Flint and KJ Sales**	**Oral Session 3: Ovary and Oocytes** (Room C) **O17** Cell adhesion and cytoskeletal architecture during early follicle growth in the mouse ovary – J Mora **O18** Sudden changes in exposure to environmental chemicals perturbs ovarian development – MR Amezaga **O19** The antrums of ovine ovarian preovulatory follicles are severely hypoxic – JF Murray **O20** Changes in granulosal and thecal expression of bone morphogenetic protein (BMP) / activin binding proteins during antral follicle development in cattle – PG Knight **O21** Assessing the effects of biological variability on follicle counting of the neonatal mouse ovary using computer-generated virtual ovaries – A Skodras **O22** Mitochondrial distribution in PUFA treated bovine oocytes – WF Marei **Chairs: GM Kidder and RJ Webb**
1230 - 1400	**Lunch (Dining Hall) (SRF AGM (1230 – 1300))**		
1400 - 1530	**SRF Student Prize** (Bernard Sunley Lecture Theatre) **O23** Molecular and functional characterization of a testis-specific TRS4 gene in spermatogenesis – AYB Tang **O24** Appropriate imprinting of H19 in extraembryonic tissues (EET) of in vitro produced (IVP), bi- and mono-parental sheep embryos – A Fidanza **O25** Identifying the targets of IL-6 ligand action in the human fetal ovary – SL Eddie **O26** Effect of prenatal androgenization on androgen receptor expression and follicle formation in the fetal sheep ovary – F Comim **O27** In vitro follicle culture: does enhanced oxygenation improve growth and development? – JM Connolly **O28** Thrombospondin-1 (TSP-1) inhibits angiogenesis and promotes follicular atresia in a novel in vitro angiogenesis assay – SA Garside **Chairs: AJ Childs and M Laird** The Postgradoc election will follow this session		
1530 - 1700	**Tea/Coffee Break with Poster Session (Marquee)**		
1700 - 1800	**Distinguished Scientist** (Bernard Sunley Lecture Theatre) The role of A-Kinase Anchoring Protein 121 (AKAP121) in the regulation of the steroidogenic acute regulatory protein and steroid hormone biosynthesis - DM Stocco **Chair: HM Picton**		
1930	**Pre Dinner Drinks (JCR Bar)**		
2000	**Conference Dinner (Dining Hall)**		

Programme – Tuesday 14th July

TIME	SESSION DETAILS
0900 - 1030	**SSR / SRF New Investigator** (Bernard Sunley Lecture Theatre) SSR New Investigator – HH Yao Testicular Dysgenesis: Fetal origin of adult reproductive deficiencies? SRF New Investigator – KJ Sales Cyclooxygenase enzymes and Prostaglandin receptors: Insights into mechanisms and control of uterine pathology **Chair: DRE Abayasekara**
1030 - 1100	**Tea/Coffee Break (Marquee)**
1100 - 1300	**Symposium 3: Non-transcriptional Modifications** (Bernard Sunley Lecture Theatre) Epigenetic processes in embryos and stem cells – LE Young Two families of RNA-binding proteins that regulate translation in germ cells and interact – NK Gray Nuclear remodelling in spermiogenesis: the histone code may survive chromatin condensation – D Miller **Chair: CJ Ashworth**
1300 - 1310	**Closing Ceremony** (Bernard Sunley Lecture Theatre) DRE Abayasekara
1310 - close	**Lunch (Dining Hall) and Depart**

Biographies

Marshall Medallist - Professor Twink Allen

Twink Allen graduated from Sydney Veterinary School in 1965 and completed a PhD degree under the supervision of Professor Roger Short FRS at Cambridge Veterinary School in 1970. He then founded, and remained until its closure in December 2007, Scientific Director of The Equine Fertility Unit a small, specialised veterinary research group based initially at The Animal Research Station in Cambridge and, after 1988, at purpose-built premises at Mertoun Paddocks in Newmarket. He was elected to the Jim Joel Professorship of Equine Reproduction and awarded an ScD degree by Cambridge University in 1995, elected to a Professorial Fellowship of Robinson College in 1996, achieved Diplomate and Fellowship status in The Royal College of Veterinary Surgeons in 1986 and 2004 respectively, was made a Fellow of The Institute of Biology and created CBE in 2002. He has *honaris causa* doctorate degrees from the Universities of Krakow, Gent and Helsinki. Since his retirement from the Cambridge chair in December, 2007 he has become Scientific Director of the newly-founded Paul Mellon Laboratory of Equine Reproduction in Cheveley Park, Newmarket where he is completing and writing up the results of the last 2-3 years experimental work of The Equine Fertility Unit and undertaking new research using archived EFU samples.

Distinguished Scientist - Dr Doug Stocco

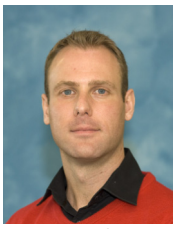
Dr. Stocco received a B.Sc. in 1967 and a M.Sc. in 1969 from the University of Windsor in Canada. He received a Ph.D. from the University of Toronto in 1972 and performed two years of postdoctoral research at UCLA. In 1974 he joined the faculty of the Texas Tech University Health Sciences Centre where he is currently a Professor in the Department of Cell Biology and Biochemistry and the Executive Vice President for Research for the TTUHSC. Dr. Stocco's research career has focused on the mechanisms involved in the regulation of steroid hormone synthesis. During the course of this research, his laboratory identified and characterized a novel protein, named the Steroidogenic Acute Regulatory or StAR protein, which is indispensable in the biosynthesis of steroid hormones. Dr. Stocco has published over 200 peer-reviewed original scientific articles, book chapters and review articles. In recognition of his work, Dr. Stocco received an NIH Research Career Development Award in 1985, and an NIH MERIT Award in 1996. He was named a Grover E. Murray Distinguished Professor in 1997 and was awarded the Robert A. Welch Endowed Chair in Biochemistry in 1998. In 2003 he was named a University Distinguished Professor. Dr. Stocco was awarded the Research Award by the Society for the Study of Reproduction in 1997, the Achievement Rewards for College Scientists (ARCS) Distinguished Scientist Award for 1997 and was also presented with the Transatlantic Medal Award by the British Endocrine Society in 1999. In 2001 he was named a Raine Distinguished Professor at the University of Western Australia. In 2005 Dr. Stocco was awarded the Dean's Research Award, the President's Research Award and the Chancellor's Council Distinguished Research Award and was elected as a Fellow of the AAAS.

Biographies

SSR New Investigator - Dr Humphrey Yao

Humphrey Yao is an Assistant Professor in the Department of Veterinary Biosciences at University of Illinois at Urbana-Champaign in USA. He received his B.S. in Biology at Fu-Jen University in Taipei, Taiwan in 1989 and then served in the military for two years. In 1994, he came to the US for his graduate study with Dr. Janice Bahr at University of Illinois. He obtained his Ph.D. in 1999 studying follicular development in chickens and completed his postdoctoral training in mechanisms of sex determination with Blanche Capel at Duke University. In 2003, Dr. Yao became a faculty member in the Reproductive Biology Program at the University of Illinois at Urbana-Champaign. Dr. Yao was the recipient of the 2004 March of Dimes Basil O'Connor Starter Scholar Research Award, 2004 Levine Junior Faculty Research Award, 2007 Pfizer Research Award, and 2008 SSR New Investigator Award. The research topics in Dr. Yao's lab include genetic regulation of ovary formation, mechanisms of testicular dysgenesis, effects of endocrine disruptors on fetal gonad development, and regulation of steroidogenic cell lineage specification. These research projects are funded by March of Dimes Birth Defects Foundation and National Institute of Health. Dr. Yao was the invited speaker for the Gordon Research Conference, North American Testis Workshop, and International Symposium for Sex Differentiation, and International Congress of Comparative Endocrinology.

SRF New Investigator - Dr Kurt Sales



Dr Kurt Sales obtained his B.Sc degree in Biochemistry and Physiology, with his honours in Pharmacology and M.Sc in Biochemistry from the University of Cape Town, South Africa. His PhD, which was awarded in 2002, was conducted in collaboration between the University of Cape Town and the Medical Research Council (MRC) Human Reproductive Sciences Unit in Edinburgh. In 2001 Dr Sales was appointed as a Post Doctoral research fellow, under the mentorship of Dr Henry Jabbour at the MRC Human Reproductive Sciences Unit in Edinburgh and was promoted to Senior MRC Investigator Scientist in 2005. Dr Sales's research has focussed on the role of cyclooxygenase (COX) enzymes, their bioactive products, prostaglandins (PG) and prostaglandin receptors in pathologies of the female reproductive tract. He has had a very active research career and co-supervised numerous PhD students and Post Doctoral fellows. During 2001-2008, Dr Sales published 17 research articles in high impact journals in addition to 7 review articles and book chapters, including seminal work in Cancer Research in 2002 where he elaborated the role of COX-1, previously thought to perform housekeeping functions, in regulating the pathology of cervical cancer. Since then he has focussed his research on the role of the specific prostaglandin G-protein-coupled receptors, EP2 and FP receptor in endometrial adenocarcinoma. Dr Sales's work has highlighted novel signal transduction pathways mediating the role of prostaglandin signalling in angiogenesis in endometrial adenocarcinomas, as well as signal transduction pathways regulating tissue architecture, and motility.

Biographies

Symposium 1: Lifestyles and Reproduction

Dr David Gems

David Gems was a postdoc at the University of Missouri-Columbia with Prof. Don Riddle before moving to UCL with a Royal Society fellowship in 1997. Much of his work uses the nematode *C. elegans* to understand the genes and mechanisms that control aging. He has also contributed to studies of aging in *Drosophila,* the mouse and the nematode *Strongyloides ratti*, and penned articles on the ethics of aging research. He is a founder member and Deputy Director of the UCL Institute of Healthy Aging, and has contributed to over 60 research papers, review articles and book chapters.
Web links: http://www.ucl.ac.uk/~ucbtdag/ http://www.ucl.ac.uk/~ucbtdag/iha/

Selected recent publications
1) R. Doonan, J.J. McElwee, F. Matthijssens, G.A. Walker, K. Houthoofd, P. Back, A. Matscheski, J.R. Vanfleteren, D. Gems. 'Against the oxidative damage theory of aging: Superoxide dismutases protect against oxidative stress but have little or no effect on lifespan in C. elegans.' Genes and Development (2008) 22: 3236-3241.
2) D.S. Patel, A. Garza-Garcia, M. Nanji, J.J. McElwee, D. Ackerman, P.C. Driscoll, D. Gems. 'Clustering of genetically defined allele classes in the Caenorhabditis elegans DAF-2 insulin/IGF-1 receptor.' Genetics (2008) 178: 931-946.
3) J.J. McElwee, E. Schuster, E. Blanc, M.D. Piper, J.H. Thomas, D.S. Patel, C. Selman, D.J. Withers, J.M. Thornton, L. Partridge, D. Gems. 'Evolutionarily conservation of regulated longevity assurance mechanisms.' Genome Biology (2007) 8, R132.

Professor Twink Allen

Twink Allen graduated from Sydney Veterinary School in 1965 and completed a PhD degree under the supervision of Professor Roger Short FRS at Cambridge Veterinary School in 1970. He then founded, and remained until its closure in December 2007, Scientific Director of The Equine Fertility Unit a small, specialised veterinary research group based initially at The Animal Research Station in Cambridge and, after 1988, at purpose-built premises at Mertoun Paddocks in Newmarket. He was elected to the Jim Joel Professorship of Equine Reproduction and awarded an ScD degree by Cambridge University in 1995, elected to a Professorial Fellowship of Robinson College in 1996, achieved Diplomate and Fellowship status in The Royal College of Veterinary Surgeons in 1986 and 2004 respectively, was made a Fellow of The Institute of Biology and created CBE in 2002. He has *honaris causa* doctorate degrees from the Universities of Krakow, Gent and Helsinki. Since his retirement from the Cambridge chair in December, 2007 he has become Scientific Director of the newly-founded Paul Mellon Laboratory of Equine Reproduction in Cheveley Park, Newmarket where he is completing and writing up the results of the last 2-3 years experimental work of The Equine Fertility Unit and undertaking new research using archived EFU samples.

Dr Fiona Denison

Dr Denison is currently a Clinical Lecturer in Obstetrics and Gynaecology at Edinburgh University.

After graduating BSc (Hons I) and MBChB (Hons), she was awarded a Clinical Training Fellowship during which time she was awarded an MD for her studies on the role of inflammation during parturition. Following a further period of clinical training, she was appointed as a Clinical Lecturer in 2004. Her current research interests lie in the impact of maternal obesity on pregnancy outcome with particular interests in vascular and placental biology. She is due to take up a Senior Clinical Lecturers position at Edinburgh University in July with one of her key clinical roles being to co-lead the metabolic antenatal clinic which provides antenatal care to women with a BMI>40.

Biographies

Symposium 2: Communication in Pregnancy

Dr Gerald Kidder

Dr. Kidder launched his research career in developmental biology with a doctorate from Yale University. After a brief research and teaching stint at Reed College in Oregon, he moved to Canada to take up an assistant professorship at the University of Western Ontario in London, Ontario where he remains to this day. He is currently appointed as Professor of Physiology, Obstetrics and Gynaecology, and Paediatrics in the Schulich School of Medicine and Dentistry, and serves as the University's Associate Vice-President (Research). He also chairs the Developmental Biology Program of the Children's Health Research Institute in London, Ontario. Through most of his career his research has utilized mice to explore mechanisms of preimplantation embryogenesis, focussing on the roles of ion transport systems and connexins (gap junction proteins) in blastocyst development. In 1995 his research team, along with collaborators at the University of Toronto, were the first to generate a connexin knockout mouse. Since then, Dr. Kidder and his research trainees have made extensive use of genetically altered mice to gain knowledge about the functions of different connexins in spermatogenesis, oogenesis, and embryogenesis. In recent years they have extended this research to the IVF clinic where they have been exploring the relationship between gap junctional intercellular communication within the ovarian follicle and oocyte/embryo quality as reflected in pregnancy outcome.

Professor Ashley Moffett

Ashley Moffett qualified as a doctor after studying in Cambridge and London and, after junior jobs in general medicine, she moved to Cambridge to train as a clinical histopathologist. Her expertise was in reproductive pathology and from this she developed an interest in the diseases of pregnancy that result from failure of placentation. She left clinical medicine and moved to the Dept of Pathology in Cambridge to work with Prof Charlie Loke. Together they described the distinctive natural killer (NK) cells present in the uterine lining during placentation. Since then she has investigated the unusual expression of MHC ligands expressed by trophoblast cells and defined how uterine NK cells recognise and respond to them. At present her group is studying women with pregnancy disorders such as pre-eclampsia to see how the defective placentation may depend on inherited combinations of maternal NK receptor genes and fetal MHC ligands.

Dr Amanda de Mestre

Mandi de Mestre is currently a Lecturer in the Department of Veterinary Basic Sciences at the Royal Veterinary College, University of London. Following completion of her veterinary degree at the University of Sydney in 1998, Mandi worked as a veterinary surgeon in equine specialist practice in Australia and the UK. In 2005 she completed her PhD in Medical Sciences in the Department of Immunology and Genetics at the John Curtin School of Medical Research, Australian National University, for which she was the recipient of the Frank Fenner Medal. This was followed by postdoctoral training in the laboratory of Professor Douglas Antczak at Cornell University where she developed her current research interests that focus on the role of the trophoblast cell in modulating immunity during pregnancy. During these postdoctoral studies, she showed that ectopically transplanted trophoblast cells had a similar lifespan in pregnant and non-pregnant mares, suggesting that trophoblast defence mechanisms are essential to protection of the semiallogeneic fetus. Mandi recently established her own laboratory at the Royal Veterinary College. Her laboratory is currently investigating the interactions that occur between equine binucleate trophoblast cells and adaptive immunity during pregnancy, and the molecular mechanisms that regulate placental development in the horse.

Biographies

Symposium 3: Non-transcriptional Modifications

Professor Lorraine Young

After initial studies on the endocrinology of Reproduction in the Universities of Aberdeen and Liverpool, Lorraine was introduced to embryology by Ian Wilmut's team at Edinburgh's Roslin Institute. By establishing that both the in vivo and in vitro preimplantation embryo is vulnerable to epigenetic changes that can have profound effects on later fetal and adult development, and demonstrating that profound epigenetic defects are found in most "cloned" embryos, Lorraine became interested in the vulnerability of human pluripotent cells to similar effects. Now Director of STEM (Wolfson Centre for Stem Cells, Tissue Engineering and Modelling) at the University of Nottingham, Lorraine's team continues to unravel the potential consequences of human embryonic and iPS stem cell epigenetic defects, how to minimise them to generate a safer and more uniform cell product and how to harness them to model human disease. She has a keen interest in training the next generation of stem cell researchers, with STEM running an MSc in Stem Cell Technology and Regenerative Medicine Doctoral Training Centre. Current projects include: 1) Scale up of human pluripotent stem cells 2) Stem cell screening of human nutrient-gene interactions at the epigenetic level 3) Switching Pluripotent Genes on and Off.

Dr Nicola Gray

Nicola Gray completed her PhD at the European Molecular Biology Laboratory in Heidelberg in 1994, focusing on the post-transcriptional regulation of gene expression. She then carried out postdoctoral research at the University of Wisconsin, Madison, where she developed an interest in translational regulation during gametogenesis and early development. In 2000, she became a Group Leader at the MRC Human Genetics Unit in Edinburgh and recently (2008) moved her laboratory to the MRC Human Reproductive Sciences Unit where she remains associated with the University of Edinburgh. Her research addresses the mechanisms by which mRNA translation is regulated by specific RNA-binding proteins, and the contribution of these regulatory events to gametogenesis, development and host-defence mechanisms.

Dr David Miller

1989-1999: Lecturer in School of Medicine at University of Leeds (UoL) from, working on heat shock proteins[1] and RNA carriage[2-3] of mature spermatozoa as well as the causes and early detection of preterm labour[4], developing new techniques in prenatal diagnosis via fetal cell recovery by trans-cervical[3] and maternal blood sampling[5].
1999-2008: Senior Lecturer in Reproduction and Early Development Group, UoL, focusing on complexity of RNA carriage in human spermatozoa relating to male fertility and on chromatin packaging in human and murine spermatozoa[6].
2008-present: Reader in Molecular Andrology, UoL, continuing work on nucleic acids carriage and packaging in the mature spermatozoon and their delivery to the egg[7-8]

1. Miller D, Brough S, Al-Harbi O (1992) *Hum Reprod* **7**, 637-45.
2.Miller D, Tang PZ, Skinner C, Lilford R (1994) *Hum Reprod* **9**, 864-9.
3. Miller D, Briggs D, Snowden H, Hamlington J, Rollinson S, Lilford R, Krawetz SA (1999) *Gene* **237**, 385-392.
4. Miller D, Briggs J, Rahman MS, Griffith-Jones M, Rane V, Everett M, Lilford RJ, Bulmer JN (1999) *Hum Reprod* **14**, 521-31.
5. Miller D, Briggs J, Rahman MS, Griffith-Jones M, Rane V, Everett M, Lilford RJ, Bulmer JN (1999). *Hum Reprod* **14**, 521-31.
6. Li Y, Lalancette C, Miller D, Krawetz SA (2008) *Asian J Androl* **10**, 535-41.
7. Ostermeier GC, Miller D, Huntriss JD, Diamond MP, Krawetz SA (2004). *Nature* **429**, 154.
8. Miller D, Ostermeier GC (2006) *Hum Reprod Update* **12**, 757-767.

Symposia Speaker Abstracts

Symposium 1: Lifestyles and Reproduction
Bernard Sunley Lecture Theatre
Sunday 12th July, 1400 – 1600

Notes

Age and reproductive fitness: evolutionary theory and biogerontological practice

D Gems
Institute of Healthy Ageing, and G.E.E., University College London, London, UK

Two key questions about ageing are why does it happen and how does it happen? The evolutionary theory provides a clear answer to the why question. Ageing evolves not because it enhances fitness, but merely because the late life effects of genes experience a weaker force of natural selection. Moreover, because of pleiotropy, genetic variants may increase reproductive success early in life, but have deleterious effects later in life, and such variants may increase fitness overall and therefore accumulate in populations (1). By contrast, the how of ageing remains one of the great unanswered questions in biology. A popular theory is that it is caused by accumulation of molecular damage (particular oxidative damage caused by reactive oxygen species), and controlled by somatic maintenance processes (mainly detoxification, repair and turnover).

In principle, the answer to the why could provide clues about the how. However, the evolutionary theory has proved to be a rather poor guide in this respect. For example, its prediction of the non-programmed nature of ageing generated the view that single gene mutations with large effects on ageing should not exist (2, 3). In fact, the discovery of such mutations founded the field of lifespan genetics. Another prediction from the evolutionary theory is that limited resources might lead to trade offs between reproduction and somatic maintenance, and that such resource allocation is a critical determinant of aging. Although reproductive can clearly decrease lifespan, the importance of somatic maintenance here remains unclear (4). Moreover, number of recent studies suggest the oxidative damage theory of ageing is incorrect (5, 6). Thus, a reappraisal of resource allocation models of ageing seems warranted.

1. Williams, G. C. (1957) *Evolution* **11,** 398. 2. Partridge, L., Gems, D. (2002) *Nat Rev Genet* **3,** 165. 3. Partridge, L., Gems, D. (2006) *Trends Ecol Evol* **21,** 334. 4. Partridge, L., Gems, D., Withers, D. J. (2005) *Cell* **120,** 461. 5. Perez, V. I., Van Remmen, H., Bokov, A., Epstein, C. J., Vijg, J., Richardson, A. (2009) *Aging Cell* **8,** 73-5. 6. Doonan, R., McElwee, J. J., Matthijssens, F., Walker, G. A., Houthoofd, K., Back, P., Matcheski, A., Vanfleteren, J. R., Gems, D. (2008) *Genes Dev.* **22,** 3236.

Physiological and man-made influences on fertility in thoroughbred horses

WR 'Twink' Allen
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Nowadays in commercial Thoroughbred studfarms popular stallions have 'books' of 140+ mares which they must try to impregnate by natural mating within the 4 month 'official covering season' (mid-Feb to mid-June) that does not match closely the physiological breeding season of the mare (mid-April – mid-September). Despite many important scientific and veterinary advances over the past 40 years, including artificial lighting to hasten seasonal cyclicity, prostaglandin analogues to induce luteolysis, synthetic oral, injectable and intravaginal progestagens to mimic dioestrus and/or supplement progesterone production during pregnancy, ultrasound scanning to monitor follicular development and ovulation, accurately diagnose early singleton and twin pregnancy to enable successful treatment of the latter and diagnose uterine pathology, GnRH analogues to hasten and synchronise ovulation with mating, and ecbolic drugs like oxytocin to treat post-mating intrauterine fluid accumulation in older mares, in which early pregnancy (i.e. days 14-42 after ovulation) loss rates rise steeply from the mean of 8% for the overall population. Equine stud veterinary clinicians are nowadays having to work harder to try to maintain the previous *conception rate per cycle* and *pregnancy rate per season* figures of 67% and 93% respectively due to subfertility in the overused stallions and the strong commercial pressure to keep breeding from older, valuable (i.e. 'black-type') mares suffering a range of age-related reproductive tract pathologies which include degenerative endometrosis and blocked oviducts. Introduction of the presently banned reproductive technologies, artificial insemination (AI) and embryo transfer (ET), would ease this burden greatly.

Impact of obesity on maternal reproductive fitness **Notes**

F Denison
Reproductive and Developmental Sciences, Simpson Centre for Reproductive Health, University of Edinburgh, Edinburgh, UK

The increasing prevalence of obesity has prompted the World Health Organisation to describe obesity as one of the most important global health problems. The rising prevalence of obesity is particularly marked in women of reproductive age reaching 20.6% in Scotland by 2003 and increasing from 11.4% to 18.5% in England. There is a high prevalence of obesity in the infertile population with obesity contributing to menstrual cycle irregularities, anovulation and reduced fecundity. The increased risks associated with obesity continue during pregnancy with pregravid obesity being associated with increased risk of morbidity and mortality for both mother and offspring. For the mother, these risks include development of gestational diabetes, hypertensive disorders including pre-eclampsia and peri-partum complications including caesarean section, thrombo-embolic events and post-partum haemorrhage. Moreover, in the most recent Confidential Enquiry into Maternal and Child Health (CEMACH), more than half of women who died were overweight or obese (BMI>25 kg/m^2) with over 15% being severely (BMI>40kg/m^2) obese. For the offspring, risks include congenital anomaly, late fetal death, admission to the neonatal unit and propensity to become obese themselves in childhood and early adolescence. Obesity is therefore a major public health problem for both non-pregnant and pregnant women and has considerable implications for provision and delivery of reproductive health services.

This symposium lecture will cover the general effects of obesity on maternal reproductive fitness before focusing on its adverse consequences for pregnancy and putative underlying mechanisms by which these adverse consequences are affected.

Symposia Speaker Abstracts

Symposium 2: Communication in Pregnancy
Bernard Sunley Lecture Theatre
Monday 13th July, 0830 – 1030

Notes

Intercellular communication in oogenesis: implications for pregnancy

G Kidder
Department of Physiology & Pharmacology, University of Western Ontario, Ontario, Canada

Introduction: Gap junctional coupling among ovarian granulosa cells is important for oogenesis since its deficiency in mice leads to developmentally incompetent oocytes and impaired folliculogenesis. Previous work had identified connexin43 (Cx43) as the major constituent of gap junctions in mouse cumulus and mural granulosa cells. The aim of this study was to determine which connexin(s) predominates in human cumulus cells and to explore a possible relationship between connexin expression, intercellular coupling strength, and pregnancy.
Methods: Cumulus cells were obtained with informed consent from IVF patients undergoing intracytoplasmic sperm injection (ICSI). Connexin expression was examined by RT-PCR and confocal microscopy. Cx43 was quantified by immunoblotting and gap junctional coupling was measured by patch-clamp electrophysiology and/or dye injection. Embryos were scored by standard morphological criteria and pregnancy was confirmed by 40 day ultrasound.
Results and discussion: Although other connexins are present, Cx43 was uniquely found to form numerous, large gap junctions between cumulus cells. The strength of gap junctional conductance varied both between patients and between individual follicles of a patient and was significantly and positively correlated with Cx43 level. Cx43 level and intercellular conductance were positively correlated with embryo quality, and were significantly higher in patients who became pregnant than in those who did not. Thus Cx43 is a major contributor to gap junctions in human cumulus cells and its expression level during oogenesis, as well as the strength of gap junctional intercellular coupling that it provides, may be an important determinant of a successful pregnancy.

How does the maternal immune system respond to trophoblast?

A Moffett
Pathology, University of Cambridge, Cambridge, UK

The trophoblast cells of the placenta form the physical interface between the mother and the fetus. They are, therefore, the fetal cells that are exposed to the potentially damaging immune system of the mother. Because of their complement of paternal genes, trophoblast cells are genetically different or allogeneic and must avoid immune rejection. Pregnancy is the only natural situation where allogeneic cells are in direct contact in mammals and we are also exploring the idea that the immune cells in the uterine lining (decidua) – composed predominantly of Natural Killer (NK) cells – can have a physiological role in influencing the success of placentation. We are particularly interested in whether the outcome of interaction between NK receptors and trophoblast Major Histocompatibility Complex (MHC) ligands leads to control of the invasion of trophoblast cells into the uterus. This type of immune response is quite unique to pregnancy.

Trophoblast-maternal interactions: what can we learn from the mare? **Notes**

AM de Mestre [1], D Hanlon [2] & DF Antczak [3]
[1] The Royal Veterinary College, University of London, London, UK; [2] Matamata Veterinary Services, Matamata, New Zealand; [3] Baker Institute for Animal Health, Cornell University, Ithaca, NY, USA

Although it is well established that trophoblast-maternal interactions play a key role in modulating immunity during pregnancy, the relative contribution of trophoblast cells, uterine factors and hormones of pregnancy remains an area of debate. The late implantation of the equine conceptus and the highly antigenic characteristics of equine invasive trophoblast cells make the pregnant mare a good model species for studies of early placental development and maternal anti-fetal immune responses. We have assessed the importance of trophoblast specific defence mechanisms to the development of fetal-maternal tolerance using transplants of invasive trophoblast cells into fully allogeneic, non-pregnant recipient mares. These studies demonstrated that transplanted trophoblast cells differentiate, function, and exert profound effects on the reproductive physiology of recipients in ectopic sites outside the uterus. Furthermore, the transplanted trophoblast cells survive in the face of strong humoral and cellular immune responses directed against the trophoblast cells. Transplanted trophoblast cells have similar lifespans in non-pregnant and pregnant mares, suggesting trophoblast derived factors are key to defence against maternal immune destruction. In order to better understand the immune reactivity of lymphocytes surrounding the invasive trophoblast cells, we have characterised the phenotype and cytokine profile of lymphocytes isolated from the endometrial cups in normal horse pregnancy. As determined by flow cytometric analysis, the percentage of IFNG+ lymphocytes was significantly increased, IL4+ lymphocytes were decreased, and IL-10+ lymphocytes were unchanged compared to peripheral blood lymphocytes isolated from the same mare. However, the percentage of CD4+FoxP3+ cells surrounding the endometrial cups was increased by 3-fold in the uterus. These findings indicate that local lymphocyte reactivity is modulated at the equine trophoblast-maternal interface. We are currently using an in vitro trophoblast/lymphocyte co-culture system to directly assess the role trophoblast specific mechanisms play in regulating these changes in immune function.

Symposia Speaker Abstracts

Symposium 3: Non-transcriptional Modifications
Bernard Sunley Lecture Theatre
Tuesday 14th July, 1100 – 1300

Notes

Epigenetic processes in embryos and stem cells

L Young
Centre for Biomolecular Sciences, University of Nottingham, Nottingham, UK

Although almost all of the cells within an individual have the same gene sequence, lineage specific gene expression patterns are established by the addition of a range of epigenetic modifications to DNA and associated histone proteins that influence transcriptional potential. Remodelling the sperm and the oocyte into pluripotent cells capable of forming all fetal lineages requires substantial remodelling of the epigenome. Reprogramming somatic cells back to a pluripotent state also requires removal of somatic cell epigenetic modifications and reactivation of the pluripotent epigenome. Our laboratory is interested in unravelling how pluripotent epigenetic states are established and how environmental factors can induce plasticity in this process that may have developmental consequences for both embryos produced via natural conception and those produced by ART. In addition our work is shedding light on the potential impact of current methods of human pluripotent cell culture on the potential biosafety of human embryonic and induced pluripotency stem cells. Our recent progress towards these goals will be presented.

Two families of RNA-binding proteins that regulate translation in germ cells and interact

NK Gray [1], JW Smith [1], B Gorgoni [1], L McCracken [1], B Richardson [1], JP Sousa Martins [1] and B Collier [2]
[1] MRC Human Reproductive Sciences Unit, Queens Medical Research Institute, Edinburgh, UK; [2] MRC Human Genetics Unit, Institute of Genetics and Molecular Medicine, Western General Hospital, Edinburgh, UK

Impaired fertility affects approximately 15% of couples worldwide with a significant proportion of cases being due to defective gametogenesis. Gametogenesis is a highly complex process that requires the exquisite temporal, spatial and amplitudinal regulation of gene expression at multiple levels. Translational regulation is important in a wide variety of cell types but may be even more prevalent in germ cells, where periods of transcriptional quiescence necessitate the use of post-transcriptional mechanisms to effect changes in gene expression. One predominant mechanism of controlling the translation of mRNAs during gametogenesis is via changes in the length of the poly(A) tail at the 3'end of specific mRNAs. These changes occur in a strict temporal manner with an increase in poly(A) tail length generally being associated with translational activation. Bioinformatic analysis suggests that approximately 40% of oocyte mRNAs in mammalian species are subject to this form of regulation. However, not all mRNAs that are translationally activated at different times during oogenesis and spermatogenesis are regulated by changes in poly(A) tail length. These mRNAs are considered to be activated by the action of mRNA specific translational activators, only a few of which have been identified to date. We will present data on one family of proteins, which we have shown to be translational activators, the Deleted in Azoospermia (DAZ) family. Members of this family have important roles in gametogenesis in a wide variety of species, and each member of this family has been linked to infertility in humans. We will discuss our findings on how these proteins regulate translation and their links to the proteins which mediate the effects of poly(A) tail length changes, the Poly(A)-binding proteins (PABPs).

Nuclear remodelling in spermiogenesis: the histone code may survive chromatin condensation

Notes

D Miller [1], DE Iles [1], MH Brinkworth [2] & A Arpanahi [1]
[1] University of Leeds, Leeds, UK; [2] University of Bradford, Bradford, UK

Following meiosis, the spermatids of many unrelated species, including the human undergo a dramatic cellular reorganisation that includes the loss of the majority of the cytoplasm accompanied by the shutdown and super-condensation of the paternal genome. Sperm-specific super-condensation during spermiogenesis is facilitated by a genome-wide repackaging of the paternal genome, largely replacing somatic and sperm-specific histones (nucleosomes) with smaller, arginine-rich protamines (nucleotoroids). The resulting nuclear chromatin of the mature spermatozoon is at least ten fold more compact than the chromatin of round spermatids and is enveloped within a nucleus that also contains distinctive RNAs. Despite the higher order packaging, paternal chromosomes adopt preferred locations within the nucleus as assessed by FISH studies. Moreover, nucleosomes are retained in the sperm nucleus as a minor to significant component, depending on species, estimated at between 1 and 50% of total chromatin. Recent CGH-chip, ChIP-chip and ChIP-sequencing strategies suggest that in human and murine sperm, some of these nucleosomes carry specific histones and histone modifications including acetylation and methylation marks in a more open conformation than the bulk protamine packaged DNA and package distinctive gene regulatory sequences with a highly specific ontology that are delivered to the egg. The delivery of sperm-specific histones and modified histones to the egg suggests that a higher level of epigenetic modification beyond the known gene imprinting marks exists in paternal chromatin that may have important implications for the developing embryo.

Post Doc Scientist Award Session

Bernard Sunley Lecture Theatre
Sunday 12th July, 1630 – 1800

Post Doc Scientist Award Abstracts

Notes

O1 Androstenedione does not overcome transforming growth factor-β1 inhibition of FSH receptor mRNA expression in cultured bovine granulosa cells.

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Centre de Recherche en Reproduction Animale (CRRA), Faculté de médecine vétérinaire, Université de Montréal, Québec, Canada

Introduction: We have recently shown that TGF-β1 inhibits androgen conversion to estradiol (E_2) in FSH-stimulated bovine granulosa cells by reducing *Fshr* and *Cyp19a1* mRNA expression and CYP19A1 activity. The general objective of this study was to identify potential endocrine factors, which may be involved in overcoming the inhibitory effects of TGF-β1 in bovine granulosa cells. Since androgens have recently been shown to stimulate *Fshr* expression in granulosa cells of growing bovine antral follicles, we hypothesized that androstenedione may be a factor that could relieve the negative effects of TGF-β1 on *Fshr* expression. We also hypothesized that androstenedione could decrease endogenous TGF-β1 action by inhibiting *Tgfb1* mRNA expression in granulosa cells.
Methods: Bovine granulosa cells from 2-5 mm follicles were cultured in serum-free medium for 6 days. To study the effect of androstenedione on TGF-β1 inhibition of FSHr expression, graded doses of androstenedione: 0, 1, and 100 nM were added on Day 0 in the presence of 0.5 ng/ml of TGF-β1 and 1 ng/ml of FSH and expression of *Fshr* was determined on Day 6 by RT-PCR. The effect of graded doses of androstenedione on *Tgfb1* mRNA expression was also examined.
Results and discussion: Androstenedione caused a significant increase in *Fshr* mRNA expression, but in the presence of TGF-β1, *Fshr* mRNA expression remained inhibited even in the presence of high doses of androstenedione. *Tgfb1* mRNA was expressed in cultured bovine granulosa cells but androstenedione did not cause any significant change in the expression of *Tgfb1* mRNA. This study shows that androstenedione cannot overcome the marked inhibitory effects of TGF-β1 on *Fshr* expression, suggesting that activation of TGF-β1 receptors may be linked to persistent inhibition of FSHR. This study also shows that endogenous *Tgfb1* expression is not regulated by androstenedione. Supported by NSERC of Canada.

O2 Dynamic changes in the expression and functionality of BMP signalling accompanies the transition from germ cell proliferation to differentiation in the human fetal ovary

AJ Childs [1], H Kinnell [1], C Collins [1], K Hogg [1], RAL Bayne [1] and RA Anderson [2]
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Introduction: Bone Morphogenetic Proteins (BMPs) are essential for specification and maintenance of primordial germ cells (GCs). However, possible roles for BMPs in regulating early meiotic GC behaviour have not been widely explored. We therefore investigated the expression and functions of BMP-signalling components in the human fetal ovary, from GC proliferation, through meiotic entry, to assembly of the first primordial follicles.
Methods: Ovaries were dissected from human fetuses (8-20 weeks (wk) gestation) obtained with informed consent following termination of pregnancy, and either frozen (for qRT-PCR, grouped as 8-9wk, 14-16wk and 17-20wk with n=5-6 per group), fixed (for immunohistochemistry), or cultured (whole (10 days) or disaggregated (24 hours) n=3-6) with or without 100ng/ml BMP4. Cultured tissues were recovered for qRT-PCR analysis or fixed for stereological determination of GC number.
Results and discussion: Transcriptional profiling of BMP signalling components revealed a switch in ligand and mediator expression coincident with the transition from GC proliferation (8-9wk) to meiotic differentiation (14-16wk), with significantly decreased expression of *BMP4*/*SMAD5* and increased expression of *BMP2*/*SMAD1*. Immunolocalisation of BMP receptors 1a and 1b and phosphoSMAD1/5/8 identified GCs to be the targets of BMP action, and demonstrated a striking shift in pSMAD1/5/8 subcellular localisation from the nucleus to cytoplasm on differentiation, revealing the existence of oocyte-intrinsic factors that negatively regulate BMP-signalling. Culture of whole 9wk fetal ovaries with BMP4 for 10 days reduced GC number and significantly decreased GC density by 41% (P=0.01), challenging the hypothesis that BMP4 promotes post-migratory GC proliferation/survival. Conversely, treatment of 14-16wk ovaries with BMP4 induced Activin betaA subunit transcription and significantly increased Inhibitor of Differentiation (*ID1-4*) gene expression, highlighting these factors as possible downstream effectors of BMP4 action. These data demonstrate the presence of a developmentally-regulated BMP signalling system in the human fetal ovary, and evolving requirements for BMP action as GCs undergo differentiation.

O3 Abnormal development of mouse preantral follicles in vitro **Notes**

SA Stubbs, A Skodras, S Franks & K Hardy
IRDB, Imperial College London, London, UK

Introduction: Isolated unilaminar follicles cannot be grown in vitro. An alternative approach is to culture intact neonatal ovaries, which contain large numbers of primordial and primary follicles. The aim of this study was to compare mouse follicle development in vitro and in vivo.
Methods: Pairs of ovaries (n=6) from C57Bl/6 pups (day 4 pp) were cultured for 8 or 12 days on floating filters ± growth factor-reduced Matrigel, in DMEM/F12 containing Albumax, BSA, selenium and transferrin. Cultured and in vivo ovaries (days 4 and 12; n=6) were fixed, paraffin embedded, sectioned and H&E stained. Health, developmental stage and dimensions of follicles were assessed in the middle section of each ovary. Relative positions of primordial and growing follicles to each other and to the ovary surface were quantified by measuring their xy coordinates and calculating Euclidian distances.
Results and discussion: Matrigel had no effect on follicle development, so cultured ovaries were pooled for subsequent analysis. On day 4 60% of oocytes were in germ cell nests. Ovaries shrank in vitro, although the classic gradient of small cortical follicles to larger medullary follicles was maintained. Follicle formation and oocyte growth occurred both in vivo and in vitro. Atretic follicles were rare in vivo but common (~20%) in the medulla of cultured ovaries. Mathematical modelling of inter-follicle communication showed common features in vivo and in vitro which were modulated by culture. The culture conditions failed to support formation of columnar granulosa cells (GCs), multiple layers of GCs and development of a theca layer, and nuclei in larger oocytes (>30 µm) were degenerating. The cultured mouse ovary therefore provides a useful model for examining key cellular processes in follicle development, which could be impaired by lack of blood supply, increased mechanical restraint due to tissue shrinkage, or failure to express key regulatory growth factors.

O4 Prostaglandin synthesis and signalling in the porcine endometrium and conceptus

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Department of Mechanisms of Hormone Actions, Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, Olsztyn, Poland

Introduction: The role of oxytocin (OT) in controlling prostaglandin F2α (PGF2) secretion during luteolysis is not as well defined in pigs as in ruminants. OT concentrations in porcine uterine lumen increase markedly during pregnancy, especially on days 11-12. Tumor necrosis factor (TNF) stimulates PGF2 secretion in vitro from luminal epithelial cells (LE) of porcine endometrium collected at luteolysis. Nevertheless, TNF mRNA is expressed in endometrium during early pregnancy. The objective of these studies was to determine the effect of OT and TNF on prostaglandin E2 synthase (mPGES-1) and prostaglandin F2 synthase (PGFS) expression and PG secretion by LE. Moreover, we determined if PGE2 receptor (PTGER2 and PTGER4) expression is affected by pregnancy status.
Methods: Endometrium samples were collected from cyclic and pregnant gilts on days 9-15. LE cells were isolated from uteri (n=9) on days 11-12 of pregnancy and incubated for 24 h with OT (100 nM) and TNF (10 ng/ml). Expression of prostaglandin synthases and PGE2 receptors was determined by Real-Time PCR and Western blot. PG concentrations were studied by EIA.
Results and discussion: TNF stimulated mPGES-1 mRNA and protein expression ($p<0.05$) and PGE2 release ($p<0.01$) by LE on days 11-12 of pregnancy. OT tended to increase mPGES-1 expression ($p=0.07$) and stimulated PGE2 secretion in pregnancy ($p<0.01$). OT and TNF had no effect on PGFS and PGF2 secretion. Endometrial content of PTGER2 protein was higher on days 11-12 of pregnancy when compared to days 11-12 of the estrous cycle ($p<0.05$) but PTGER4 expression was not affected by pregnancy status. These studies suggest an important role of TNF and OT in regulation of PGE2 synthesis in LE during early pregnancy. The effect of increased PGE2 synthesis during days 11-12 of pregnancy may be enhanced by up-regulation of PGE2 receptors (PTGER2).

(Supported by SCSR grant N311319135. A.W. is funded by the Foundation for Polish Science.)

SRF Student Prize Session

Bernard Sunley Lecture Theatre
Monday 13th July, 1400 – 1530

SRF Student Prize Abstracts

Notes

O23 Molecular and functional characterization of a testis-specific TRS4 gene in spermatogenesis

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Introduction: Spermatogenesis is a process in which diploid spermatogonia undergo mitotic, meiotic divisions and cellular differentiation to produce haploid spermatozoa that are capable to fertilize an egg. To understand the molecular mechanisms on how spermatogenesis in rodents is regulated, we investigated the gene expression of heat-treated testis in a rat model and identified TRS4 as a heat-sensitive gene in testis. Mouse TRS4 gene is located at chromosome 6A 3.1 with 13 exons, which encodes a protein with a predicted molecular weight, 90 kDa. Bioinformatic analysis showed that TRS4 has a high sequence homology among rat, mouse and human. TRS4 protein possesses an ubiquitin domain near N-terminus and an IQ-calmodulin binding motif inside exon 4-6 region of the gene.
Methods: In this study, we aimed: (i) to study the spatiotemporal expression of TRS4 mRNA in mouse tissues and post-natal mouse testes by quantitative PCR; (ii) to study interacting partners of TRS4 protein in the testis; and (iii) to generate TRS4 conditionally knockout mice by gene-targeting approach.
Results and discussion: Quantitative PCR studies showed that TRS4 mRNA was specifically expressed in the testis and post-natally on Day 20 onward. TRS4 mRNA was also localized at the spermatids stage of seminiferous tubules of adult mouse testes by *in-situ hybridization*. By co-immunoprecipitation and Western blotting, TRS4 was found to interact with β-actin, but not VAD1.2 and VAD1.3 (acrosome-expressing proteins), or syntaxin 1. TRS4 conditional gene targeting vector was constructed by flanking the exon 4-6 region of TRS4 gene with two LoxP sites. The Cre/Flp recombination of this completed vector was characterized *in vivo* by 293-Cre and 293-Flp *E.Coli* cells respectively. Putative TRS4 targeted mouse ES clones were being screened by Southern Blotting. Results from the present study should shed light to understand the role of TRS4 in spermatogenesis.

[This work is supported in part by NSFC/RGC grant to KFL]

O24 Appropriate imprinting of H19 in extraembryonic tissues (EET) of in vitro produced (IVP), bi- and mono-parental sheep embryos

A Fidanza, P Toschi, N Pasquariello, A Pizzuto, F Zacchini, M Maccarrone, P Loi & G Ptak
Department of Biomedical Science, University of Teramo, Teramo, Italy

H19 gene is the paternally imprinted gene in which the CpG–rich promoter region becomes methylated postzygotically, upon implantation in mice. We wanted to examine first, when the methylation of the paternal *H19* gene is established in extraembryonic tissues (EET) in sheep. Bisulphite modification of DNA extracted from EET obtained from natural pregnancies on day 18, 20, 22 and 24 of embryo development (day 16= implantation starts) revealed that the paternal copy becomes progressively methylated, with fully methylated status acquired by day 22. Our preliminary studies (presented on this meeting) show that, the development of fully in vitro produced embryos (from oocyte maturation till blastocyst stage), including those monoparental, is associated with a perturbed *H19* gene expression and general developmental delay on day 22 (assessed by foetal gross morphology and crown-rump length). Thus, we wanted to know also, whether or not this perturbation is reflected by a delayed timing of the acquisition of methylation marks on an imprinted gene *H19*. The results show similar to controls timing of methylation (day 22) in majority of IVP biparental EET. In 14% of them the absence of methylated form, likely consequent to in vitro production procedures, was noted. In monoparental EET, two maternal *H19* alleles were both undermethylated on day 22, as expected. In conclusion, this results show that notwithstanding of highly deregulated mRNA expression, an appropriate methylation of paternally imprinted gene *H19* occurs in majority of in vitro produced embryos.

O25 Identifying the targets of IL-6 ligand action in the human fetal ovary **Notes**

SL Eddie [1], AJ Childs [2], RAL Bayne [2], HN Jabbour [2] & RA Anderson [1]
[1] Centre for Reproductive Biology, University of Edinburgh, Edinburgh, UK; [2] Human Reproductive Sciences Unit, Medical Research Council, Edinburgh, UK

Introduction: Interleukin 6 (IL-6) type cytokines signal through complexes of ligand-specific receptors and the common gp130 subunit to activate JAK/STAT signalling pathways and regulate cellular processes. IL-6 type ligands (including IL-6 and leukaemia inhibitory factor (LIF)) have important roles in reproduction and development, including promoting germ cell survival *in vitro*, regulating the primordial to primary follicle transition and controlling ovulation (in rodent models). We therefore investigated the expression of IL-6 type signalling components in the human fetal ovary to determine possible effects on maturation/development.
Methods: Ovaries were dissected from human fetuses (8-20 weeks) obtained with informed consent following medical termination of pregnancy, and snap frozen (for RNA) or fixed (for immunohistochemistry). Expression of *IL-6*, *LIF*, and their receptors was assayed by RT-PCR. Immunohistochemistry for gp130 and the ligand-specific receptor LIFR was used to determine spatiotemporal expression of ligands and receptors in fetal ovarian tissue.
Results and discussion: Expression of mRNA for *IL-6, LIF* and their common receptor *gp130* was detected in the human fetal ovary at all gestations. Quantitative RT-PCR analyses across a range of gestations are underway to determine whether expression of these factors is developmentally regulated.
Immunohistochemistry for gp130 indicated that it was expressed exclusively by germ cells in second trimester ovaries. As gp130 is required for signalling by all IL-6 type ligands, this result demonstrates that germ cells are the target of these cytokines in the human fetal ovary. Consistent with this, LIFR was also predominantly localised to the germ cells. The LIF signalling complex consists solely of these two components, LIFR and gp130, indicating that LIF action in the fetal human ovary is germ cell specific. These data provide a basis for roles for IL-6 type cytokines in the direct regulation of germ cells in the developing human ovary prior to primordial follicle formation.

O26 Effect of prenatal androgenization on androgen receptor expression and follicle formation in the fetal sheep ovary

F Comim [1], SA Stubbs [1], R Forsdike [1], J Robinson [2], K Hardy [1], S Franks [1]
[1] Institute of Reproductive and Developmental Biology, Imperial College, London, UK; [2] Institute of Comparative Medicine, University of Glasgow, Glasgow, UK

Introduction: Prenatal exposure to excess androgen results in abnormalities of preantral follicle development in adult sheep, similar to those seen in women with polycystic ovary syndrome (PCOS) suggesting an important role for androgens in early follicle development. The aim of this study was to investigate the effect of prenatal androgenisation (PA) on establishment of the primordial follicle pool in the fetal sheep ovary.
Methods: Pregnant sheep received either no treatment (controls) or testosterone propionate 100mg im twice weekly between days 30 and 90 of gestation. Pregnancy was terminated at day 90 and fetal ovaries removed. Ovarian sections from fetal sheep (6 androgenised and 6 control fetuses) were examined. A specific marker, VASA, was used to identify germ cells by immunohistochemistry (IHC). H and E staining was used to evaluate follicle formation (ie to calculate the proportion of germ cells enclosed within primordial follicles). Androgen receptor (AR) protein expression was assessed by IHC.
Results and discussion: 7246 germ cells and 1969 follicles were identified in 12 ovarian sections. The proportion of germ cells enclosed in primordial follicles in the ovarian sections from PA fetuses (mean (SD)) was 38.8 (13.1) % compared with 19.8 (3.8) % in control animals ($p=0.024$; Student's t test). AR was abundantly present in granulosa cells (GCs) and stroma of ovaries in both controls and PA sheep, but a higher proportion of GCs were AR+ in PNA animals ($p=<0.0001$).
In summary, AR was more abundantly expressed in granulosa cells and the ovaries of PA fetuses had twice as many germ cells that were enclosed in follicles compared with ovaries from control fetuses. These findings suggest that the increased pool of preantral follicles in women with PCOS may be due, at least in part, to exposure to excess androgen during prenatal development.

Acknowledgements: MRC (UK); CAPES (Brazil).

Notes

O27 In vitro follicle culture: does enhanced oxygenation improve growth and development?

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In vitro ovarian follicle culture is a tool used to investigate the processes that control folliculogenesis. Work from our laboratory has shown that the standard 5% CO2 in air gas phase (20% O2) does not supply sufficient O2 to support optimal development of *in vitro* cultured mouse ovarian follicles. The central hypothesis here is that optimal delivery of oxygen to follicles *in vitro* improves follicular growth and development.
Preantral 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 5% female mouse serum, 1 IU/ml FSH and 25 µg/ml ascorbic acid. Follicles were treated with superoxide dismutase (SOD; 100 IU/ml), Trolox (100 µM) or haemoglobin (10, 100 mg/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 analysis. hCG (1.5 IU/ml) was added to medium on day 5 of culture and ovulation rates were assessed.
Follicles cultured in 40% O2 had significantly greater ($P < 0.01$) terminal diameters, improved growth and ovulation rates, and enhanced oestradiol secretion in comparison to those in 20% O2. Addition of haemoglobin to follicles cultured in 20% O2 significantly improved growth ($P < 0.01$) compared to controls. At 40% O2, 10 mg/ml haemoglobin significantly improved follicle growth ($P < 0.05$) and ovulation rate ($P < 0.01$). Trolox and SOD had no effect on follicle growth.
From these data it is concluded that follicles cultured under conditions of improved oxygenation, as achieved by culture in 40% O2 with haemoglobin supplementation, exhibit improved growth and development. The beneficial effects of haemoglobin may be attributed to improvement in oxygen delivery to follicles. However, its function as a nitric oxide scavenger must also be considered.

O28 Thrombospondin-1 (TSP-1) inhibits angiogenesis and promotes follicular atresia in a novel *in vitro* angiogenesis assay

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Introduction: Ovarian folliculogenesis is regulated by many endocrine, paracrine and autocrine factors, and as follicular development progresses, increased angiogenesis is essential to sustain development of the rapidly expanding follicle. Angiogenesis is the growth of new blood vessels from existing vasculature and it is tightly regulated by a variety of pro- and anti-angiogenic factors, including vascular endothelial growth factor (VEGF) and thrombospondin-1 (TSP-1). Aberrant angiogenesis occurs in a number of pathological conditions, such as polycystic ovary syndrome (PCOS) and endometriosis, and novel ways of targeting this could lead to new treatments. TSP-1 is an anti-angiogenic factor in many physiological systems, but its role in follicular angiogenesis and development is unclear. We have developed a novel culture system to study the effect of TSP-1 on follicular angiogenesis and development.
Methods: Intact preantral/early antral follicles from 21-day-old rat ovaries were cultured for six days in the presence of TSP-1 (0, 1, 10, 100 and 1000 ng/ml). At the end of the culture period, angiogenic sprouting from the follicles was quantified using image analysis. Follicles were fixed and sectioned and follicular apoptosis was assessed by immunohistochemistry for activated caspase-3. In a subsequent experiment, isolated granulosa cells from 21-day-old rat ovaries were cultured with TSP-1 (0, 10, 100 and 1000 ng/ml) for 2 days, and apoptosis was quantified using a luminescent caspase-3/7 assay.
Results and discussion: The results showed that TSP-1 inhibited follicular angiogenesis ($p<0.01$) and promoted follicular apoptosis ($p<0.001$) in a dose-dependent manner. TSP-1 also promoted apoptosis of isolated granulosa cells in a dose-dependent manner ($p<0.01$), suggesting that TSP-1 can act independently of the angiogenesis pathway to promote follicular apoptosis. Increased angiogenesis and decreased atresia are hallmarks of PCOS. TSP-1 treatment could potentially have a dual effect by targeting the abnormal angiogenesis in the polycystic ovary and by facilitating the destruction of abnormal follicles via induction of apoptosis.

Distinguished Scientist Session

Bernard Sunley Lecture Theatre
Monday 13th July, 1700 – 1800

Distinguished Scientist Abstract

The role of A-Kinase Anchoring Protein 121 (AKAP121) in the regulation of the steroidogenic acute regulatory protein and steroid hormone biosynthesis

Notes

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Steroid production in steroidogenic tissues increases rapidly in response to tropic hormone stimulation, an acute process that is in large part regulated by the steroidogenic acute regulatory protein (StAR). StAR mediates the transfer of cholesterol across the mitochondrial membranes and it has been demonstrated that acute steroidogenesis has an indispensable requirement for the action of StAR at the mitochondrial membrane. In steroidogenic tissues StAR expression and activity are tightly controlled by cAMP-dependent protein kinase (PKA), which phosphorylates both transcriptional activators of the *Star* gene and the StAR protein itself. As a result of these mechanisms, steroidogenesis is highly sensitive to cAMP, and minimal concentrations of cAMP appear both necessary and sufficient to support acute steroidogenesis. We hypothesized that such sensitivity is in part due to PKA anchoring proteins (AKAPs) that organize and enhance the effects of cAMP signaling by selectively tethering either Type I or Type II PKA to discrete subcellular compartments within the cell. Since both subtypes of PKA are present in Leydig cells, which we use as a model system, we have examined how cAMP analog pairs that preferentially activate each PKA subtype impact StAR synthesis and activity in MA-10 mouse Leydig tumor cells. Both subtypes of PKA appear to enhance discrete events coordinating StAR-mediated steroidogenesis. Type I PKA strongly increased StAR expression as well as the phosphorylation of hormone sensitive lipase (HSL). Intriguingly, cholesterol trafficking may also impact steroidogenesis through StAR, as HSL activity and lipoprotein-induced effects were observed to increase the expression of StAR in response to cAMP. In contrast, Type II PKA was responsible for the post-transcriptional regulation of StAR expression and phosphorylation. Corroborating this observation, we also find that PKA compartmentalization and StAR-mediated steroidogenesis are enhanced by AKAP121. This mitochondrial AKAP appears to tether Type II PKA and possibly *Star* mRNA to the mitochondria, and we hypothesize that AKAP121 enhances the post-transcriptional expression and activation of STAR protein at the outer mitochondrial membrane. We anticipate that determining the specific roles that Type I and Type II PKA serve in regulating StAR will not only improve our understanding of how steroidogenesis is controlled, but may also shed light on signal transduction networks that are distinct or permissive for acute steroidogenesis.

(Supported by NIH Grant HD17481 and the Robert A. Welch Foundation Grant # B1-0028).

SSR and SRF New Investigator Award Session

Bernard Sunley Lecture Theatre
Tuesday 14th July, 0900 – 1030

SSR and SRF New Investigator Abstracts

Notes

Testicular Dysgenesis: Fetal origin of adult reproductive deficiencies?

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Although they initially form as simple tubular structures, the fetal testis cords undergo extensive elongation and coiling prior to birth. Despite the dramatic nature of this transformation, little is known about the mechanisms behind this process. Activin A, a protein product of the inhibin beta A gene (*Inhba)*, has conserved roles in epithelial-mesenchymal interactions required for the patterning of tubular structures. In the fetal testes, we observed the presence of activin A in the interstitium outside the testis cords whereas P-SMAD2/3, the downstream effector of activin signaling, was localized to Sertoli cells. This expression pattern suggests that interstitium-derived activin A could act upon the Sertoli cell epithelium during testis cord development. Utilizing the Cre/loxP conditional knockout (cKO) scheme, we removed *Inhba* in most somatic cells of the mouse testes. The testis cords of *Inhba* cKO embryos formed normally but failed to coil properly. This impairment in coiling resulted from decreased Sertoli cell proliferation. To confirm that Sertoli cells are the direct targets of interstitium-derived activin A, we removed *Smad4*, a downstream component of activin signaling, in a Sertoli-specific manner. As in *Inhba* cKO embryos, the testes of newborn *Smad4* cKO mice exhibited dysgenic testis cords with reduced cord convolution and significantly decreased Sertoli cell proliferation. To evaluate whether the dysgenic testis cords present in fetal life underwent compensatory development postnatally, we analyzed young adult mice between 12-16 weeks of age. In both testis-specific *Inhba* and Sertoli-specific *Smad4* cKO mice, there was a significant reduction in testis weight and daily sperm production. However, these cKO mice were virilized normally, with anogenital distance and seminal vesicle weight similar to controls. Histological examination of testes from adult testis-specific *Inhba* and Sertoli-specific *Smad4* cKO mice revealed multiple abnormalities including increased seminiferous tubule diameter and failure of spermiation. Taken together, these findings demonstrate that interstitium-derived activin A acts upon the fetal Sertoli cells through Smad4 to promote testis cord expansion and coiling. Disruption of this pathway results in fetal testis cord dysgenesis that persists into adulthood, leading to reduced testis size as well as impaired sperm production. (Supported by NIH-HD46861 and ES07326).

Cyclooxygenase enzymes and Prostaglandin receptors: Insights into mechanisms and control of uterine pathology

KJ Sales
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Cyclooxygenase (COX) enzymes catalyze the rate-limiting biosynthesis of prostaglandins (PG). Following biosynthesis PG exert an autocrine/paracrine function by coupling to specific G-protein coupled receptors.
In cervical and endometrial carcinomas, we have established that expression of COX enzymes (COX-1 and COX-2), PG (PGE_2 and $PGF_{2\alpha}$) and PG receptors (EP2/EP4 and FP receptor) are elevated in neoplastic epithelial cells and endothelial cells of the microvasculature compared with normal tissue. Using an in vitro HeLa Tet-Off system to inducibly express COX-1 or Ishikawa cell line stably expressing COX-2, we found that elevated COX enzyme expression up-regulated the biosynthesis of PG and induced the expression of PG receptors. Coincident with the up-regulation of PG and PG receptors, we also observed up-regulation of potent angiogenic factors and down-regulation of anti-angiogenic factors. These findings lead to the suggestion that targeted inhibition of COX enzyme may be of potential benefit as therapeutic regimens for cervical and endometrial carcinomas. However, such therapeutic strategies have now been abandoned by Pharmaceutical companies due to the adverse cardiovascular side effects of specific COX enzyme inhibitors.
Subsequently we have elucidated the signal transduction pathways mediating the role of specific PG via their receptors in cervical and endometrial carcinomas. For example, activation of the FP receptor in endometrial cancer cells promotes rapid cytoskeletal reorganization, formation of focal adhesion complexes and integrin-extracellular matrix engagement to promote cancer cell adhesion and migration. Coincident with these dynamic changes in cell behaviour, FP receptor signaling also promotes the production of potent angiogenic factors such as VEGF and FGF2 to facilitate vascular branching and sprouting and endothelial cell proliferation and angiogenesis. Taken together, our research has highlighted the use of specific PG receptor antagonists or inhibitors of key signal transduction pathways as potential therapeutic intervention strategies to inhibit the adverse effects of elevated PG signaling in uterine carcinomas.

Oral Session 1: Reprogramming

Bernard Sunley Lecture Theatre
Monday 13th July, 1100 – 1230

Oral Session 1 Abstracts

O5 Role of P2X receptors in the activation of anion secretion in the bovine oviduct epithelium **Notes**

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Introduction: Fluid within the oviduct provides the appropriate environment essential for gamete transport, fertilization and early embryo development. At this time very little information is available on the underlying mechanisms of oviduct fluid formation. Previous studies from our lab highlighted a role for basolateral P2Y purinoceptors in promoting chloride secretion by bovine oviduct epithelial cells (BOECs). The present study explored the role of P2X receptors in apical ATP-stimulated anion secretion.
Methods: Ionic secretion was measured by short-circuit current (ISC) across voltage-clamped polarised oviduct epithelial cell monolayers. Receptor expression was assessed by RT-PCR using gene specific primers.
Results and discussion: Apical ATP (100µM) stimulates a rapid transient increase (28.82 +/- 1.66 µA/cm2) in anion secretion in bovine oviduct epithelial cells, followed by a sustained increase in current. In the presence of amiloride the transient component is preserved and the sustained component significantly increased. This suggests ATP may have a dual effect, inhibiting amiloride sensitive I_{SC} while concurrently activating amiloride insensitive anion secretion. A number of P2X specific agonists rapidly activated anion secretion with a potency profile of ATP=2MeSATP> , meATP>BzATP. The lack of specificity of the available agonists means the exact purinoceptor subtype cannot be deciphered, however these data highlight multiple functional P2X receptors in BOECs. Furthermore, RT-PCR analysis using specific primers for $P2X_1$, $P2X_2$, $P2X_3$, $P2X_4$, $P2X_5$, $P2X_6$ and $P2X_7$, confirmed the expression of the $P2X_4$, $P2X_5$, and $P2X_7$ subtypes in BOECs. Considering the cross-desensitization of the ATP response to UTP, and the lack of cross-desensitization of ATP to , meATP, suggests these agonists stimulate distinct receptor subtypes. In conclusion these results demonstrate the functional expression of multiple P2X receptors in the apical membrane of BOECs, which when activated rapidly promote anion secretion, providing more evidence that ATP is likely to play an important role in fluid formation in the oviduct optimizing conditions for fertilization.

O6 Human cumulus cell gene expression as a biomarker of pregnancy outcome following single embryo transfer

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Introduction: Embryo developmental potential is reliant on the quality of the gametes the embryo is derived from. A marker of oocyte and embryo developmental potential would augment successful pregnancy outcomes following ART by optimising oocyte and embryo selection. The direct communication linking the oocyte and its surrounding cumulus cells indicate a role for the cumulus cells as a marker of oocyte health.
Methods: Following oocyte retrieval cumulus cells were collected, and all oocytes and resulting embryos were cultured and transplanted singly. Gene expression was analysed in cumulus cells from independent oocytes that yielded a successful term pregnancy (n=12) compared to those for which pregnancy failed (n=26). Real-time RT-PCR assays quantitated against standard curves of known plasmid copy number for each target were run to assess gene copy number relative to two internal controls.and correlated to pregnancy outcomes. Matched pairs of cumulus cells from eleven patients were assessed to determine a correlation between cumulus gene expression associated with oocytes which resulted in a high quality vs poor quality embryo.
Results and discussion: Cumulus cell gene expression positively correlated with successful pregnancy outcome following single embryo transfer. Specifically, *PTGS2* and *VERSICAN* mRNA expression was significantly ($p < 0.05$) higher in cumulus cells from oocytes that achieved a live birth, while *PTX3* mRNA expression showed a trend towards significance ($p = 0.066$). No significant differences were seen correlated with clinical embryo grade. The results of this study support the hypothesis that the close bi-directional communication between the oocyte and its surrounding cumulus cells mediates oocyte developmental competence and provides a unique opportunity to assess the relative potential among a cohort of oocytes to achieve successful pregnancy.

Notes

O7 Parthenogenetic activation of ovine oocytes vitrified at germinal vesicle stage

AR Moawad, J Zhu, I Choi & KHS Campbell
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Parthenogentic activation of vitrified oocytes has been reported in cattle and pig (Dinnyes *et al*., 2000; Somafi *et al*., 2006), however, there are no reports in sheep. This study was designed to compare two protocols (1) a combination of calcium ionophore, cychloheximide and cytochalasin B (CA+CHX/CB), (2) strontium and CB (Sr/CB) on activation of ovine oocytes vitrified at germinal vesicle stage.
Cumulus oocytes complexes were randomly divided into three groups (1) Untreated (control), (2) toxicity, (3) vitrified (Moawad *et al*., 2008). After 24 h *in-vitro* maturation, oocytes were activated by (5 µM CA in Hepes-SOF for 5 min and 10 µg/ml CHX + 7.5 µg/ml CB in mSOF) or (10 mM Sr + 7.5 µg/ml CB in CZB) for 4-5 h. Cleavage 24 and 48 h post-activation (pa), development to blastocyst (7 days pa), and total cell numbers were examined.
Cleavage 24 hpa (6.2 v 3.8 %) and 48 h pa (28.4 v 27.5 %) was significantly (P< 0.05) lower in vitrified oocytes activated by (CA+ CHX/CB and Sr/CB, respectively) than other groups. CA+CHX/CB significantly increased (P< 0.05) cleavage 48 hpa in both toxicity (85.7 %) and control groups (92.7 %) than Sr/CB (40 and 63.3 %, respectively). No blastocyst developed from vitrified oocytes activated by CA+CHX/CB; however, 3.8 % developed from Sr/CB oocytes. This percent was significantly lower (P< 0.05) than toxicity and control (20 and 27.3 %, respectively). No significant difference was observed in the proportion of hatched blastocysts between Sr/CB vitrified, toxicity and control groups (1.3 v 5 and 4.3 %, respectively) or in total cell numbers (85 v 89.6 and 105.6, respectively).
This is the first report of parthenogentic development of ovine oocytes vitrified at GV-stage. In conclusion, strontium can be used effectively for parthenogenetic activation of vitrified immature ovine oocytes.

O8 Comparison of the behavioural responses of Yellow Coat Colour A^{vy}/a Mice to Brown Coat Colour (Pseudoagouti) A^{vy}/a Sibling Mice

CS Rosenfeld [1], DA Warzak [2], J Mao [1] & PT Sieli [1]
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Viable yellow (A^{vy}) mice, which have an intracisternal A particle (IAP) embedded in the agouti gene, are a classic animal model to study epigenetics. Yellow coloured offspring develop diabetes, obesity, and, in certain strains, cancer, but their darker siblings with brown coat colour remain healthy, even though all the mice are on the same C57Bl6J background and almost genetically identical. Whilst the weight gain and diabetic responses in these mice have been described, no previous study has compared behavioural responses of yellow coat colour mice to their brown coat colour (pseudoagouti) siblings. We compared behavioural responses of 4-5 month old yellow (n=30) to pseudoagouti C57Bl/6J (n=11) siblings by using an ActivityPro open-field maze, which tracks movement, periods at rest, total distance traveled, stereotypical behaviours, and speed. Activity data was accumulated for 15 min and analyzed by ANOVA. These studies were approved by our ACUC committee. Female and male yellow coat colour mice weighed more (40.8±10.4 and 41.9±7.2g, respectively) than female and male pseudoagouti siblings (24.3±4.2 and 31.6±1.6g, respectively, P<0.01). Female yellow coloured mice remained stationary longer, had reduced speed, and were less likely to turn counter-clockwise than pseudoagouti sisters (P<0.05). Their speed was negatively correlated with increased body weight in yellow females (P<0.001). Yellow coloured males remained stationary in the center longer, traveled less distance and at reduced speed, and were less likely to go counterclockwise than pseudoagouti brothers (P<0.05). These responses were negatively correlated with increased body weight in yellow males (P<0.05). In summary, heavier yellow coloured mice remain stationary longer and are more sluggish than pseudoagouti counterparts. Similar to humans, a vicious cycle might exist in these mice with increased obesity underpinning less activity, which further increases weight gain. Future studies will test whether diet alteration in these mice increases behavioural activity and correspondingly decreases their body weight.

O9 Simplifying sheep androgenetic embryo production **Notes**

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Androgenetic embryos are useful model for investigating the contribution of paternal genome to embryonic development. We previously established a procedure for producing diploid androgenetic sheep embryos through the fertilization of enucleated oocyte and pronuclear transfer (Wakayama et al., 2007). Pronuclear transfer was necessary for the development to blastocyst stage of diandric sheep embryos was very poor (1%, Wakayama et al., 2007). We envisaged that one reason for the poor development of androgenotes might have been the pre-activation of oocytes during the enucleation step. Therefore, we set up preliminary experiments to verify whether oocytes undergo spontaneous activation following the enucleation. In vitro matured oocytes (85) were enucleated in TCM199 medium supplemented with Cytocalasin B and 5% BSA, and checked for pronuclear organization 10-15 hour after; a control group was kept in the same culture condition without manipulation (43). More than half of the oocytes showed spontaneous activation induced by the manipulation procedures (48/85 – 56%), whereas very few in the control group displayed activation (2/43 – 0.4%). We next compared the developmental potential of androgenetic embryos produced with pronuclear transfer with those produced through in vitro fertilization of oocyte enucleated in Calcium, Magnesium free enucleation medium to avoid spontaneous activation. At 22 h of maturation, oocytes with extruded first polar body were enucleated in PBS ($Ca^{++}Mg^{++}$ free) + BSA (8 mg/ml) with Hoechst33342 and cytochalasin B (7.5 g/mL) under UV light. Enucleated oocytes were *in vitro* fertilized (IVF); normally fertilized embryos were produced as control. After IVF, presumptive zygotes were transferred in SOF medium and cultured till blastocyst stage.
Androgenetic blastocyst rate did not differ significantly between the experimental groups (15.80% vs 18.80% for $Ca^{++}Mg^{++}$ free medium and pronuclear exchange respectively). In conclusion, these findings demonstrate the feasibility to produce diploid androgenetic sheep embryos using a simple protocol with fertilization of enucleated oocytes.

O10 Induced pluripotent stem cells derived from pigs

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The purpose here has been to create porcine pluripotent stem cells that can expand the use of swine as a biomedical model for studying human disease, particularly for testing the efficacy and safety of transplants derived from such cells. Despite reports of the creation of embryonic stem cells from porcine blastocysts and epiblast over the past 20 years, none of the cell lines have met the criteria required to confirm pluripotency. Our approach has been to generate “induced” porcine pluripotent stem cells (iPPSC) by a similar strategy used recently for the mouse and human, namely ectopically expressing reprogramming genes in porcine fetal fibroblasts (PFF). Here PFF were infected with a mixture of lentiruses expressing h*OCT4*, h*SOX2*, h*KLF4* and h*c-MYC*. On d 2 after infection, cells were transferred to dishes containing monolayers of irradiated mouse embryonic fibroblasts (MEF) and medium supplemented with knock-out serum and FGF2. Compact colonies of alkaline-positive cells resembling human embryonic stem cells (ESC) began to emerge after about 25 days, which were selected and subcultured, allowing the generation of over 150 clonal lines. Selected clones were analyzed further. The cells were positive for porcine OCT4, NANOG, and SOX2 and had high telomerase activity. Unlike human ESC but like murine ESC, the iPPSC were positive for SSEA1. Transcriptional profiling on Affymetrix (porcine) microarrays and by real time RT-PCR supported the conclusion that re-programming to pluripotency was complete. One cell line, ID1 had a normal karyotype, a cell cycle time of ~17 h, and has been maintained through over 190 doublings. The ID6 line formed embryoid bodies, which expressed genes representing all three germ layers, when cultured under conditions that drove differentiation. It also formed highly differentiated teratomas containing tissues of ectoderm, mesoderm and endoderm origin when injected into nude mice. We conclude that porcine somatic cells can be re-programmed to form cells that are pluripotent.

Supported by grants from NIH (HD21896) to RMR and the Missouri Life Science Research Board to TE.

Oral Session 2: Female Reproduction

Room A
Monday 13th July, 1100 - 1230

Oral Session 2 Abstracts

Notes

O11 Effect of FSH and 17β-oestradiol on *in vitro* levels of PGE_2 and COX-2 in explants of cervical tissue from ewes in the follicular phase

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Introduction: In the cervix of the ewe there is some degree of relaxation during oestrus associated with high concentrations of ovarian steroids and gonadotrophins. It has been suggested that two of these, 17β-oestradiol and FSH, regulate, by intra-cervical mechanisms, the production of PGE_2, through an oxytocin receptor and cyclooxygenase 2 (COX-2) mediated pathway. The aim of the study was to determine the effects of FSH and 17β-oestradiol on the *in vitro* production of PGE_2 and the level of COX-2 in cervical explants from ewes in the follicular phase of the oestrous cycle.
Methods: Six Ile de France ewes were synchronized using flugestone intravaginal sponges for 12 days and culled 48 hours after sponge removal. The cervixes were divided transversely into 3 equal sections; the uterine end, the mid region and the vaginal end. Each section was finely chopped and 200 mg of chopped tissue used to establish explants cultured in 1ml of MEM. The explants were set up in replicate. After 24 hours of pre-incubation the media was exchanged, and the tissues incubated in the presence of FSH (10ng/ml) or 17β-oestradiol (1ng/ml) for further 48 hours. At the end of culture, media was collected and analysed by RIA for PGE_2. The tissue was lysed and the lysates analysed by Western Blotting for COX-2.
Results and discussion: Both FSH (61,0±7,05; P=0,05) and 17β-oestradiol (63,2±4,62; P=0,004) stimulated the production of PGE_2 compared to controls (43,8±3,45). The level of COX-2 was increased by FSH (2,40±0,32; P=0,01) and 17β-oestradiol (2,80±0,33; P=0,02) compared to controls (1,51±0,33). The interaction between region of the cervix and treatment was not significant for both PGE_2 and COX-2. The results show that FSH and 17β-oestradiol increased the levels of PGE_2 and COX-2 and suggest that FSH and 17β-oestradiol regulate cervical dilation of ewes in oestrus trough the induction of COX-2.

O12 Foetal long-term implantation with a GnRH agonist reduces the number of primordial follicles in 6 week-old female calves

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Introduction: Studies in sheep have demonstrated that the gonadotrophin increase during mid-gestation is necessary for adequate reproductive development in males but not females (Thomas et al., 1994). However, in a pilot study we found that in cattle long-term implantation with GnRH agonist from day 90 of pregnancy tended (P<0.10) to reduce the number of primordial follicles in newborn female calves. The objective of this study was to investigate the effect of continuous GnRH agonist infusion, during pregnancy on primordial follicle development and hormone patterns in female calves.
Methods: Fifteen female calves whose mothers were continuously infused (2.5ul/hr, Alzet, U.S.A.) with GnRH agonist from either day 40 (n=5) or 90 (n=6) of gestation until parturition were compared to untreated controls (n=4). Frequent blood samples, to evaluate LH pulses and FSH and oestradiol concentrations were collected at 1 and 3 weeks of age. Calves also received a 10ug i.v. injection of GnRH to measure pituitary LH response. At 6 weeks of age ovaries were obtained, fixed, paraffin-embedded and the follicle population analysed.
Results and discussion: Female calves, whose mothers received GnRH from day 40, showed a 3-fold decrease (P<0.05) in the number of primordial follicles compared to day 90 implanted and controls (25974.8±5815.1, 75800.9±11310.7 and 81106.39±13874.6 follicles, respectively). There was no effect on the pattern of LH, FSH and oestradiol, nor on the LH response to GnRH. Implantation of cows with GnRH agonist from day 40, but not from day 90, of gestation decreased the number of ovarian primordial follicles in their female offspring, but had no long term effect on gonadotrophin concentrations or the LH response of the pituitary to GnRH. It is concluded that GnRH agonist has affected oogenesis in foetuses during the early stages of sexual development, but further work is required to elucidate the mechanisms involved.

O13 Effect of n-3 polyunsaturated fatty acid supplementation on prostaglandins produced by uterine endometrial cells isolated from cyclic ewes **Notes**

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Introduction: Dietary n-3 polyunsaturated fatty acids (PUFAs) can benefit human health but their effects on reproduction are uncertain. n-3 PUFAs are the precursors of 3-series prostaglandins (PGs) whereas n-6 PUFAs give rise to 1- and 2-series PGs. Both PUFA families utilise the same enzymes to generate PGs, so supplementation with n-3 PUFAs can influence 2-series PG production. In ovine endometrium PGF_2 is produced primarily by epithelium and PGE_2 by stroma, with 2-series PGs playing crucial roles in luteolysis and parturition.
Methods: Uterine endometrial cells were harvested from early luteal phase ewes using a collagenase-trypsin digestion. Stromal and epithelial cells were separated based on their differential plating speeds and cultured to confluence in DMEM/F12 medium with 10% fetal calf serum. Cells were then cultured for 24h in serum free medium containing physiological concentrations (0, 20 or 100 μM) of the n-3 PUFAs, -linolenic acid (ALA), stereodonic acid (SDA) or eicosapentaenoic acid (EPA) in the absence (CM) or presence of 0.1ug/ml lipopolysacharride (LPS, stroma) or 250nM oxytocin (OT, epithelium). Spent medium was collected for PG radioimmunoassay. Each treatment was quadruplicate with cells from 10 ewes. Mixed model analysis tested effects of PUFA and LPS or OT challenge.
Results and discussion: LPS increased stromal PGE_2 production (CM 8.2±3.81, LPS 25.22±3.81ng/ml, $P<0.001$) but OT did not alter epithelial PGF_2 production (CM 10.7±3.03, OT 12.0±1.83 ng/ml). These response patterns were not altered by PUFA treatment. All three n-3 PUFAs increased PG production in both stromal and epithelial cells, with the greatest response to SDA (1.5 to 3-fold increase). In both cell types 20 μM EPA increased PG production significantly whereas 100 μM had no effect. These data suggest that n-3 PUFA consumption may influence reproductive processes and agreed with our previous studies in which dietary n-3 PUFA supplementation increased uterine PG production in cows.

O14 Unbalanced expression of a panel of relevant imprinted and non imprinted genes regulating placental growth and angiogenesis in sheep foetuses produced by Assisted Reproductive Technologies (ART)

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In vitro culture and manipulation of embryos can lead to aberrant placental and foetal development in all mammals studies so far. The development of a normal placental angiogenesis is central for further foetal growth. A reduced placental vascular development, associated with embryonic mortality, has been described in ART derived mice and sheep foetuses. Placental developmental and function is strongly regulated by imprinted genes, therefore, the majority of the studies have focused on their epigenetic deregulation in ART foetuses. However, placental angiogenesis relays mostly on non imprinted genes. In this work we analyzed by Real-Time PCR the expression levels of 22 imprinted and non imprinted genes, including several angiogenetic factors and components of Notch signalling pathway, which are involved in vascular development and differentiation. Extraembryonic tissues were collected at day 20 of gestation (previous studies have indicated that angiogenesis starts in this stage), from embryos obtained by natural breeding (control), parthenogenesis (PA), *in vitro* fertilization (IVF) and Somatic Cell Nuclear Transfer (SCNT). Altered levels of mRNA expression were found in the experimental embryo models comparing with the controls. Particularly deregulated were FGF2 and various components of Notch pathway like Notch-1,2,4 receptors and Dll1, a specific ligand. In addition, placenta from ART embryos showed a down regulated expression of imprinted genes, like H19, PHLDA2, IGF2, as well as a reduced expression of DNA methyltransferase-1 (Dnmt1). Our results demonstrated a deregulation of genes regulating placental development and angiogenesis, affecting both imprinted and non imprinted genes, in sheep ART derived foetuses. Remarkably, the imprinted genes whose expression we found altered are totally different from those described in mice, suggesting important differences between species in the epigenetic alteration resulting from extreme ART, such SCNT. To conclude, our work bring a significant contribution toward the molecular characterization of the ART-derived placental phenotypes, and strongly suggest that equal attention should be focused on non imprinted genes.

Notes

O15 Contractions induced by prostaglandin (PG) E_2 and $PGF_{2\alpha}$ are superseded by thromboxane in isolated human myometrium at term pregnancy

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Introduction: Prostaglandins (PG) E_2, $PGF_{2\alpha}$ and thromboxane modify uterine activity via EP, FP and TP receptors respectively (Senior *et al*., 1993). However, the underlying mechanisms are not fully understood. The aim of this study was to determine EP, FP and TP-mediated contractions relative to receptor mRNA expression and calcium (Ca^{2+}) signalling pathways in human myocytes at term pregnancy.
Methods: Lower segment myometrial biopsies were obtained at elective Caesarean section from consenting term pregnant women, not in labour (37-41 weeks gestation; n=14). Ethical approval was granted by the Local Regional Ethics Committees. To record contractility, strips of myometrium were mounted under physiological conditions and attached to isometric force transducers. Vehicle (saline) and concentration-effect curves were constructed for PGE_2, $PGF_{2\alpha}$ and U46619 (a thromboxane mimetic) [10^{-10}M to 10^{-5}M]. For Ca^{2+} assays, primary human myocytes were cultured (passages 1-3) and loaded with the Ca^{2+} indicator fluo-4AM dye (2μM); agonist responses were measured using a fluorometric imaging plate reader (FLIPR). In addition, qualitative gene expression for $EP_{1\text{-}4}$, FP and TP mRNA was detected using RT-PCR. Results were expressed as arithmetic means ± S.E.M. and analysed using ANOVA with Bonferroni's post-hoc test.
Results and discussion: Compared to vehicle controls, PGE_2 induced a biphasic response initially reducing contractions ($p<0.01$) with some excitation at 10^{-5}M. Whilst U46619 was more potent than $PGF_{2\alpha}$ in augmenting myogenic activity ($p<0.001$), exposure to $PGF_{2\alpha}$ produced greater Ca^{2+} transients in cultured myocytes ($p<0.01$). This reflected the high expression of FP mRNA, which was much more abundant than TP, EP_2, EP_1, EP_3 and EP_4 receptors ($p<0.001$).
Despite the predominant mRNA expression and Ca^{2+} release via the FP receptor, the results of this study indicate that the uterus is more responsive to TP receptor activation. This suggests that the TP receptor may enhance uterine contractions via alternative signalling pathways, which are currently being investigated.

O16 Regulation of angiogenesis and lymphangiogenesis in human endometrium via PGF2alpha-FP initiation of adrenomedullin signalling

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Introduction: We have previously shown that adrenomedullin (AM) is increased in endometrial cancer compared with normal endometrium and that AM expression is regulated by PGF2alpha. AM has been reported to play an important role in angiogenesis and cancer progression. In the present study, we investigated the expression of adrenomedullin receptor (CLR) in normal and neoplastic endometrium and the effect of conditioned medium (CM) from PGF2alpha treated FP over-expressing cells on vascular and lymphatic endothelial cells.
Methods: Endometrial tissue was obtained with full ethical approval and patient informed consent. Cell culture studies were performed using endometrial adenocarcinoma explants and the Ishikawa endometrial adenocarcinoma cell line stably expressing the FP receptor (FPS cells). Human umbilical vein endothelial cells (HUVECs) and human dermal lymphatic endothelial cells (HDLECs) were used to investigate the role of AM on endothelial cells.
Results and discussion: The adrenomedullin receptor (CLR) was localised to vascular endothelium in normal and neoplastic endometrium. Triple staining with antibodies to CLR, CD31 (general endothelial cell marker) and podoplanin (lymphatic endothelial cell marker) localised expression in vascular and lymphatic endothelial cells. Treatment of FPS cells and adenocarcinoma explants with PGF2alpha induced a significant increase in AM RNA and protein, compared with vehicle treated controls ($P<0.05$). Treatment of HUVECs or HDLECs with CM collected from PGF2alpha treated Ishikawa FPS cells or with AM peptide induced a significant increase ($P<0.05$) in proliferation, migration and network formation of HUVECs and HDLECs. In addition, these cells showed decreased permeability in response to these treatments ($P<0.05$). These effects were inhibited by two antagonists to CLR ($CGRP_{8\text{-}37}$ and $AM_{22\text{-}52}$) or by neutralizing AM from the CM ($P<0.05$).
In conclusion, we have shown that PGF2alpha -FP action regulates AM expression in endometrial cancer cells and that AM subsequently acts in a paracrine manner via its receptor CLR to promote angiogenesis and lymphangiogenesis in the human endometrium.

Oral Session 3: Ovary and Oocytes

Room C
Monday 13th July, 1100 – 1230

Oral Session 3 Abstracts

O17 Cell adhesion and cytoskeletal architecture during early follicle growth in the mouse ovary **Notes**

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Introduction: Female mammals are born with a lifetime's supply of oocytes, which remain quiescent until stimulated to grow. Regulation of this transition remains poorly understood. Quiescent oocytes are surrounded by a layer of flattened granulosa cells (GCs) forming primordial follicles. Initiation of follicle growth is accompanied by dramatic cellular changes, including oocyte growth and GC cuboidalization, proliferation and subsequent multi-layering. We hypothesize that change in GC shape is key, and that increased understanding of changes in cell adhesion and cytoskeletal architecture will provide insight into the regulation of initiation of follicle growth and help identify key regulators, and their mechanisms of action.
Methods: Ovaries were obtained from C57BL/6 mice on days 4, 12, 21 and 42 post partum. Immunofluorescence with confocal microscopy was used to examine the expression of cell adhesion molecules, as well as proteins associated with the cytoskeleton.
Results and discussion: E-cadherin, a classical epithelial adhesion molecule, was observed on the oocyte membrane of follicles at all stages of growth, but not between the GCs. Interestingly, N-cadherin was observed between GCs of all growing follicles, co-localized with the cadherin-associated protein beta-catenin. The tight junction protein ZO-1 was observed on the oocyte membrane of all follicles and on the cell membranes of adjoining cuboidal GCs. The intermediate filament protein Cytokeratin-8 was strongly expressed in the GCs of primordial follicles, but not in growing follicles. Actin expression was observed in follicles at all stages of development, particularly in transzonal processes and the theca layer. Microtubules (alpha-tubulin) were observed in a radial alignment as GCs cuboidalized and were prominent in radial processes as follicles formed multiple layers.
The expression of cell adhesion and cytoskeletal proteins changes as GCs cuboidalize and multilayer. The lack of E-cadherin, and expression of N-cadherin and cytokeratin-8 suggests that GCs are not truly epithelial.

O18 Sudden changes in exposure to environmental chemicals perturbs ovarian development

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Introduction: We have previously shown that in-utero exposure to low doses of complex mixtures of environmental chemicals (ECs) reduces oocyte and primordial follicle numbers, further, exposure from conception leads to higher proportions of unhealthy primordial follicles in 18-month-old ewes. We aimed to establish whether the timing of in-utero exposure to a mixture of ECs, either prior to, or after, conception, affects fetal development.
Methods: Treated ewes (TT, n=12) were exposed to ECs by pasturing on fields fertilised with sewage sludge whereas controls (CC, n=12) grazed on fields fertilised with inorganic fertiliser. Other ewes were transferred at mating to a different condition: controls to treatment (CT, n=12) and treatments to control (TC, n=12). At day 110 of gestation, fetal ovaries were harvested and body mass and organ weights recorded. Numbers of follicles (Lundy classification) were counted in H&E sections (48 fields of view/ovary). IHC for VASA and YBOX were performed. Fetal ovarian proteins were analysed using 2D gels followed by LC-MS/MS and Western blots (WB).
Results and discussion: While in-utero exposure to ECs had no significant effect on fetal weights, ovarian weight was significantly higher in exposed fetuses (CC=41±4g, TT=56±5g, CT=55±6g, TC=50±6g) and adrenal weight was significantly higher in TC (TC =361±29g, CC=336±26g, TT=321±30, CT=277±30). Treatment groups had fewer healthy ovarian type 3 follicles/mm^2 than controls (CC=0.3±0.1, TT=0.1±0.1, CT=0, TC=0.1±0.1). CT had fewer healthy type 1a follicles/mm^2 (CT=17±6, CC=43±4, TT=42±5, TC=32±4) and more healthy type 0 follicles/mm^2 (CT=1.9±0.7, CC=1.1±0.5, TT=0.3±0.6, TC=0.2±0.5). 49 protein spots were significantly affected by treatment, including proteins with roles in binding toxins/heavy metals, heat shock, cell cycle and cell division. HSP70 (WB) was significantly reduced in CT compared with CC. These data showed fewer prolonged exposure effects than previously reported but demonstrated marked effects of sudden changes in exposure at conception, particularly from control conditions to sewage sludge exposure.

Notes

O19 The antrums of ovine ovarian preovulatory follicles are severely hypoxic

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Introduction: The pO_2 of each compartment of the ovarian follicle has yet to be accurately determined but, importantly, the results of many studies to date consistently support the hypothesis that the centre of the follicle, where the oocyte resides, has a significantly lower pO_2 concentration than other body tissues (normally about 40mmHg). It was the aim of this study to accurately determine both pO_2 concentrations and temperatures in the antrums of preovulatory ovarian follicles.
Methods: The oestrous cycles of 12 mule ewes were synchronized. Forty-four hours after progestagen sponge removal and injections of prostaglandin $F_{2\alpha}$ analogue and PMSG, the ewes were placed under terminal anaesthesia. By laparotomy, the ovaries were exposed and an OxyLite (Oxford Optronix) probe placed in the centre of antral follicles. The OxyLite probe is both an oxygen-sensing device and a thermocouple. For each follicle both the pO_2 concentration and temperature were continuously recorded for several minutes. All results are presented as mean ± SEM.
Results and discussion: In total 33 antral follicles of varying diameter were interrogated with an OxyLite probe. Robust pO_2 concentration traces were obtained from 18 follicles. The mean pO_2 concentration from these traces was 2.6±1.1mmHg. The mean temperature of all follicles was 35.6±0.3°C which was lower than the mean core temperature (38.4±0.1°C: $P<0.001$). These results will be corroborated by immunohistochemical detection of adducts formed from the extrinsic hypoxia probe, pimonidazole, when cells experience less than 10mmHg pO_2 (all ewes were injected with pimonidazole prior to surgery). We have demonstrated that the centre of the antral follicle is very hypoxic and we have also confirmed that, as has been observed in other species, the temperature of the follicle is lower than core. We hypothesize that both hypoxia and the lower temperature are important in reducing the incidence of chromosomal abnormalities in the oocyte.

Supported by The Wellcome Trust.

O20 Changes in granulosal and thecal expression of bone morphogenetic protein (BMP) / activin binding proteins during antral follicle development in cattle

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Introduction: Evidence supports key roles for intraovarian TGFβ superfamily members including activins and bone morphogenetic proteins (BMP) in follicle development. Access of these ligands to their signalling receptors is modulated by several extracellular binding proteins. In this study we used Q-PCR to compare expression of mRNA transcripts for four secreted BMP/activin binding proteins (chordin, gremlin, follistatin, noggin) and one membrane-bound binding protein (BAMBI) in granulosal (GC) and thecal (TC) compartments of bovine antral follicles.
Methods: TC and GC layers were recovered from follicles ranging from 1-20mm in diameter and grouped into six size-classes. Total RNA was extracted, reverse-transcribed and cDNA used for QPCR amplification using target-specific primers. For each sample, transcript abundance was normalized to β-actin and resultant ΔCt values re-normalized to the average ΔCt for that transcript in all samples analysed (i.e. $\Delta\Delta$Ct). Concentrations of oestradiol (E) and progesterone (P) in follicular fluid were also determined and E:P ratio calculated. Follicles in the 11-20mm size-band were subdivided into 'E-active' (E:P ratio>1) or 'E-inactive' (E:P ratio <1).
Results and discussion: Two-way ANOVA revealed that, overall, the relative abundance of all five transcripts was higher in GC than TC ($P<0.001$). Two-way ANOVA revealed a significant ($P<0.05$) effect of follicle size-class on abundance of chordin, gremlin and follistatin mRNA, with a significant cell-type x size class interaction. With noggin and BAMBI mRNA the effect of follicle size-class was not significant ($P<0.1$) although there was a significant follicle size-class x cell type interaction for noggin ($P<0.05$) but not BAMBI ($P=0.09$) mRNA. Abundance of GC mRNA for noggin and BAMBI was lower ($P<0.05$) in 'E-active' than 'E-inactive' large follicles. In contrast, abundance of follistatin mRNA was ~10-fold higher ($P<0.01$) in the 'E-active' group. These findings support the hypothesis that extracellular binding proteins play an important role in regulating intrafollicular BMP/activin signalling.

Supported by BBSRC

Notes

O21 Assessing the effects of biological variability on follicle counting of the neonatal mouse ovary using computer-generated virtual ovaries

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Introduction: Studies of the effects of ageing, specific genes, environmental toxins and cancer therapeutics on the complement of follicles in the ovary require accurate approaches to count the total number of follicles at different stages of follicle development. A variety of techniques are used, which are generally laborious, time-consuming and involve sampling the ovary to produce estimates of follicle number. The two main factors affecting such estimates are the counting technique (sampling frequency) and biological variability, i.e. the variation of follicle numbers between animals of the same strain and age. Our study aims to assess the relative contribution of sampling frequency and biological variation to the accuracy of the estimates obtained, using 'virtual', computer-generated ovaries.
Methods: We have developed computer algorithms to generate virtual mouse ovaries, using parameters derived from biological data from 1 week-old mice. The virtual ovaries contain realistic numbers of primordial, primary and secondary follicles (themselves of realistic size), which have the classic distribution within the ovary from small follicles in the cortex to larger follicles towards the medulla. We know the number of follicles in each virtual ovary, and can compare this with estimates obtained from virtual histological sections. We use these ovaries to carry out experiments in silico, varying the number of ovaries analysed, and the frequency of sections analysed (eg 1 in every 5^{th}, 20^{th} or 50^{th} section). Furthermore we generate virtual 'wildtype' and 'knockout' ovaries (with varying numbers of follicles).
Results/Discussion: We found that analysis of a few sections (1 in 20) of more ovaries (8 or more), gave more accurate estimates than frequent sampling of fewer ovaries. We conclude that biological (between-animal) variability is the greatest source of inaccuracy in counting studies. This information allows us to plan realistic experiments, and draw meaningful and robust conclusions.

O22 Mitochondrial distribution in PUFA treated bovine oocytes

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Introduction: Polyunsaturated fatty acids (PUFAs) can influence bovine oocyte development both in vivo and in vitro. Cytoplasmic maturation, including mitochondrial reorganization, is a set of crucial changes that determine the developmental competence of the oocytes. Mitochondria are the key component of the metabolic process responsible for energy supply during oocyte maturation. We have previously reported that the type of PUFA affects oocyte nuclear maturation; -linolenic acid (ALA; n-3 18:3) significantly increases whereas linoleic acid (LA; n-6 18:6) retards/ or inhibits oocyte maturation to metaphase II stage. Here we investigated the effect of these PUFAs on mitochondrial distribution during oocyte maturation.
Methods: Oocytes (20 oocytes per treatment, 3 repeats) were denuded of cumulus cells (by vortex for 2 min), cultured for 0, 1, 3 or 24h +/- ALA (50μM) or LA (100μM) and stained with MitoTracker Green (100nM) for 30 min before fixation in paraformaldehyde (2%) containing 30 mg/ml Hoescht. Oocytes were imaged using confocal microscopy (Leica).
Results and discussion: Two patterns of mitochondrial distribution were found: (1) pericytoplasmic (mainly at 0h), and (2) diffuse. At early stages of nuclear maturation, mitochondrial clusters were observed between 1-3h of oocyte culture. Most of these clusters were perinuclear. Oocytes supplemented with ALA showed no significant differences in mitochondrial reorganization compared to the controls. Supplementation of LA resulted in: (1) delayed redistribution of the pericytoplasmic mitochondria at 1h (P=0.01); (2) a significant decrease in the percentage of the oocytes with mitochondrial clusters at 1h (27±4% vs. 63±9%; P=0.04) and 3h (38±5% vs. 57±5%; P=0.04) and (3) fewer oocytes having a perinuclear mitochondrial distribution at 1h (21±4% vs. 47±6%; P=0.01) and 3h (21±5% vs. 40±8%; P=0.004) compared to controls. Failure of the mitochondria to redistribute to form clusters and arrange around the nucleus during early maturation may therefore be an important factor inhibiting oocyte nuclear maturation in LA supplemented oocytes.

POSTERS

Notes

P1 BRomo Domain Testis-specific (BRDT) expressing fibroblasts are successfully reprogrammed to blastocyst stage after nuclear transfer

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Incomplete nuclear reprogramming is responsible for the low efficiencies in Somatic Cell Nuclear transfer (SCNT). Testis-specific factors mediating the transition from somatic/sperm-specific conformation of chromatin might be potential candidates for improving SCNT. In a preliminary study we demonstrated that mice BRomo Domain Testis-specific (BRDT) protein induces chromatin condensation in sheep fibroblasts. The aim of this experiment was to verify whether sheep somatic cells expressing of the mice BRomo Domain Testis-specific (BRDT) protein are suitable donor cells for SCNT.
Sheep skin fibroblasts were cultured in MEM plus FBS. Cells were transfected with GFP-BRDT (Green Fluorescent Protein tagged BRDT) plasmid and treated with Tricostatin A (TSA) (100ng/mL) for 16h, then cultured for 48h in 0.5% FCS before nuclear transfer. Un-transfected fibroblasts were used as control. Metaphase II oocytes were enucleated and reconstructed with control fibroblasts and BRDT positive ones, and electrofused through a DC pulse (1,20 kV/cm for 10 µs) with a BTX electroporator (ECM 830) in fusion medium. The successfully fused embryos were cultured in SOF medium with 1% (v:v) basal medium Eagle (BME) essential amino acids (Gibco), 1% (v:v) minimum essential medium (MEM) nonessential amino acids (Gibco) and 8 mg/ml BSA. Oocytes reconstructed with BRDT positive fibroblasts developed to blastocysts stage in higher proportion comparing to control ones (9,7% vs 3.6%, respectively - P< 0.01).

Experimental groups	n. oocyte reconstructed (%)	n. fused (%)	Morula stage embryos (%)	Blastocysts stage embryos (%)
BRDT	255	134	25/134 (18.65%)	13/134 (9,7) ±0.09)
Control	41	28	3/28 (10.7%)	1/28 (3.6) ±0.07)

These preliminary data demonstrated that DRDT expressing fibroblasts can be used for SCNT, and suggest that the large scale BRDT chromatin reorganisation induced by BRDT might results in a more extensive nuclear reprogramming, at least till blastocyst stage.

P2 Gene expression profiling of single human oocytes and preimplantation embryos

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Background: Although hundreds of thousands of human embryos are cultured in fertility centres across the UK each year, a vast proportion fail to develop in culture or fail to implant following transfer to the uterus. Identification of key regulatory networks during early human preimplantation development will increase our current understanding of the events that dictate oocyte competence and normal preimplantation embryo development. It will also be valuable for assisted reproductive technologies (ART) to aid identification of viable embryos for successful transfer, and for assessment of novel embryo technologies. Using a microarray based approach, our aim was to increase global understanding of the developmental competence of human oocytes and embryos, which may also be advantageous for embryo selection in clinical IVF cycles.
Methods: Single oocytes, 4-cell stage embryos and blastocysts were lysed, reverse transcribed and globally amplified by PolyAPCR. PolyAcDNA was hybridised to Affymetrix GeneChip Human Genome U133 Plus 2.0 arrays and analysed using the “Database of Annotation, Visualisation and Integrated Discovery” (DAVID).
Results and Discussion: 1476 genes were expressed in at least 2 out of 3 oocytes (P<0.05; q<0.22). Genes involved in the TGFβ signalling pathway were highly represented in all oocytes while the insulin signalling pathway was significant in only 2/3 oocytes, suggesting the third oocyte may have been developmentally compromised. Genes encoding enzymes involved in amino acid metabolism were highly represented in all blastocysts (P<0.05; q<0.22). Genes involved in the cell cycle and focal adhesion pathways were significant in only 2/3 blastocysts, suggesting the third blastocyst was developmentally compromised. Expression of purine and pyrimidine pathway components was common to oocytes and blastocysts. The further investigation of these pathways will aid our understanding of the signalling cascades involved in early human preimplantation development.

P3 Expression and imprinting analysis of the H19 Gene in human preimplantation embryos **Notes**

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Introduction: Imprinted genes are susceptible to the epigenetic disruption that is associated with *in vitro* culture of mammalian embryos. The *H19* gene is a maternally expressed non-coding transcript that may act *in vivo* as a tumour suppressor. Expression and methylation of the *H19* gene is frequently affected during *in vitro* culture of mammalian embryos. Therefore, expression, imprinting stability and methylation status of the *H19* gene may be useful as biomarkers of epigenetic disruption in human assisted reproduction. We sought to assess expression and imprinting status of the *H19* transcript in a large cohort of human preimplantation embryos derived by both standard IVF and ICSI procedures.
Methods: Heterozygous embryos were identified by sequencing or restriction enzyme digestion of *H19* genomic PCR products around transcribed *Rsa*I and *Alu*I polymorphisms in the *H19* gene. Allelic expression was then assessed in embryonic cDNA libraries by sequencing or restriction digestion of *H19* PCR products. For other embryo samples, genomic DNA was not available. Here, *H19* PCR products were amplified from cDNA libraries and analysed by sequencing or restriction enzyme digestion.
Results and discussion: *H19* expression was detected in 45 of a total of 92 tested morula or blastocyst stage embryos. Expression is stage-specific since expression was not observed in morula stage embryos or early blastocysts but was frequently detected in fully expanded blastocysts. In embryos heterozygous for at least one polymorphism, imprinted expression was detected in 4 embryos, whilst partial monoallelic expression was observed in 2 embryos. Five further embryos with partial monoallelic or biallelic expression were observed. Expression and imprinting status of *H19* is therefore variable between human preimplantation embryos. Imprinting variability was observed in both IVF and ICSI embryos. This study provides further evidence of variable genomic imprinting between *in vitro* derived human embryos however, the foundation for this observation remains to be identified.

P4 Pregnancy adversely affects cognitive function specifically spatial memory: Effects of task and stage of pregnancy

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Background: Female sex hormones influence learning and memory and the neurobiology of brain regions involved, however, it is unclear whether cognitive changes in pregnancy are due directly to these changes or indirectly through their effect on mood. The aim of this investigation is to increase understanding of the effects of pregnancy on cognition.
Methods: Participants, (n=47) pregnant (PG) and non-pregnant controls (CG) were tested each trimester and following birth. Edinburgh postnatal depression (EPDS) and general health questionnaire 12 (GHQ12) scales were administered at each session. The Cambridge Neuropsychological Test Automated Battery examined working memory, planning ability and attention. Venous blood samples were obtained at each session for quantitative hormone assessment. Data were analysed and compared using SPSS version 14. The study received ethics approval.
Results: During the 2nd and 3rd trimester and at three months following birth, pregnant participants showed a significant performance deficit in mean spatial recognition memory, 2nd trimester PG 70%±2.5, CG 82%±1.9 (p=0.0001), 3rd trimester PG 74%±2.4, CG 80%±1.8 (p=0.03) and at three months following birth, PG 66% ±2.2, CG 79%±2.1, (p=0.0001). Delayed matching to sample-mean probability error was significantly greater for the pregnant group in the 1st trimester compared to the control group, PG 0.17 ±0.03, CG 0.09 ±0.03 (p=0.04).
Compared to the control group, pregnant group mean EPDS scores were significantly higher in the 1st and 2nd trimester and GHQ12 scores significantly higher in 1st, 2nd and 3rd trimester, group scores for both measures of wellbeing were then similar at three months following birth. Group demographics were similar.
Conclusions: Data suggest pregnancy adversely affects spatial memory. Although pregnant group EPDS and GHQ12 scores are significantly higher than controls they remain within 'normal' parameters, it is unlikely that they would therefore, adversely affect memory function, but this requires further investigation. The significant finding in terms of probability error may indicate a memory processing deficit.

Notes

P5 Circulating sex hormone-binding globulin in the human fetus

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Introduction: Human plasma sex hormone-binding globulin (SHBG) is produced primarily by hepatocytes and binds testosterone and oestradiol with high affinity and selectivity to regulate transport and access of these sex steroids to their target tissues. SHBG is also expressed in different steroid sensitive tissues, including Sertoli cells, ovarian follicles and corpora lutea in both adult and fetal tissues. We aimed to determine circulating fetal concentrations of SHBG and liver expression of *SHBG* and *CYP* enzymes involved in steroidogenesis and detoxification.
Methods: Plasma and livers were collected from 58 fetuses from electively-terminated, normally-progressing, pregnancies (12-19 weeks, REC 04/S0802/21), equally divided between male and female fetuses and also according to maternal cigarette use during gestation. Fetal plasma SHBG and cotinine (to confirm cigarette exposure) concentrations were determined by ELISA. SHBG concentrations were analysed with respect to gender, gestational age, and the smoking status of the mother (ANOVA and multivariate analysis).
Results and discussion: Fetal SHBG concentrations (nmol/l) were similar between groups (male smoker: 9.2±1.1, n=16; male control: 7.9±1.2, n=14; female smoker: 8.0±1.1, n=15; female control: 6.7±1.0, n=12). While smokers tended to have higher circulating SHBG, this was not statistically significant. Fetal circulating SHBG concentrations were not different between male and female fetuses, or across the second trimester. We found no significant correlation between circulating SHBG and fetal and maternal indices such as body weight, BMI, cigarettes smoked or fetal cotinine concentrations and gonad weight. SHBG levels measured in this study were 3-fold lower than levels reported for cord blood. Studies are underway to determine if transcript expression of *SHBG* and *CYP* enzymes in the fetal livers are affected by maternal cigarette smoking. We conclude that maternal cigarette smoking does not affect fetal circulating SHBG. In addition, cord blood SHBG measurements may not be a good analogue for circulating fetal SHBG.

P6 Effects on the pituitary of chronic treatment with the GnRH agonist deslorelin

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Introduction: Gonadotropin-releasing hormone (GnRH) agonists have emerged as an important animal contraceptive and a frontline treatment for several human disorders including advanced prostate cancer and endometriosis. GnRH agonists work largely by eliminating the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary gland, yet the precise consequences of chronic GnRH agonist treatment on the pituitary remain unknown. In this study, we describe changes in the number of cells immunoreactive for LH, FSH, or both following chronic treatment with the GnRH agonist deslorelin in rats.
Methods: Approximately 125-day-old male Sprague-Dawley rats received 1.1mg (n=4) or 4.7mg (n=4) sc implants of deslorelin or received no implants (control; n=4). After 45 days, pituitaries were fixed via transcardial perfusion and sagittaly sectioned (20µm). Using standard immunocytochemical techniques, mid-sagittal sections were fluorescently labeled for LHβ and FSHβ. The number and percentage of immunoreactive cells, including those colocalizing both hormones, and the total number of cells were determined.
Results and discussion: Treatment with deslorelin significantly reduced the number and percentage of cells immunoreactive for FSHβ, but βLH-immunoreactive cells were unaffected. The number of colocalized cells was also significantly reduced, and the percentage of colocalized cells tended to differ (p=0.06). These data show that chronic GnRH agonist exposure selectively affects the FSH gonadotrope subpopulation. Additional preliminary data suggest that this effect is not through the loss of testosterone.
Procedures were approved by the University of Wyoming Animal Care Committee (IACUC #A-3126-01).
Research was supported by Grant Number RR15640 from the NCRR, a component of the NIH. AS was funded, in part, by a grant from NSF #EPS-9983278.

P7 Kisspeptin and GnRH pulse generator activity in the adult female rat **Notes**

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Introduction: Kisspeptin and its cognate receptor GPR54 are essential for activation of the hypothalamo-pituitary-gonadal axis. Kisspeptin neurones in rats are located in the hypothalamic arcuate (ARC) and anteroventral periventricular (AVPV) nuclei. Although it is generally accepted that the AVPV kisspeptin plays a critical role in the central regulation of the LH surge, it is not clear whether kisspeptin neurones in the AVPV or ARC contribute to GnRH pulse generator activity. To explore the relationship between GnRH pulse generator activity and kisspeptin-GRP54 signalling in the ARC and AVPV, we examined the effects of intra-ARC or intra-medial preoptic area (which includes the AVPV) administration of kisspeptin-10 or a selective kisspeptin antagonist on pulsatile LH secretion in the rat.
Methods: Ovariectomised rats with s.c.17β-oestradiol capsules (plasma ~38pg/ml) were chronically implanted with bilateral intra-ARC or intra-medial preoptic area (mPOA) cannulae and intravenous catheters. Blood samples (25μl) were collected every 5 min for 5 h for LH measurement. After 2 h of control blood sampling, kisspeptin-10 (1, 10 or 100 pmol, in 400nl artificial cerebrospinal fluid, aCSF) or kisspeptin antagonist (10 or 50 pmol, in 400nl aCSF; 3 injections at 30 min intervals) was administered by intracerebral injection. Animal procedures were undertaken in accordance with the United Kingdom Home Office Regulations.
Results and discussion: Central administration of kisspeptin-10 (intra-ARC or intra-mPOA) resulted in a dose-dependent increase in circulating levels of LH lasting approximately 1 h, before then recovering to a normal pulsatile pattern of circulating LH. However, intra-ARC administration of kisspeptin antagonist dose–dependently suppressed LH pulse frequency without affecting LH pulse amplitude. Intra-mPOA administration of kisspeptin antagonist did not affect pulsatile LH secretion. These data suggest that, kisspeptin-GPR54 signalling in the ARC but not the mPOA contributes to the generation or modulation of LH pulses in the rat.

(BBRSC, MRC, Wellcome Trust. YC supported by SRF Vacation Scholarship).

P8 Identification of novel proteins associated with uterine receptivity in fertile women

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Introduction: Molecular mechanisms which operate in spatio-temporal manner to endow the endometrium with receptivity are not yet elucidated in humans. This may be attributed to our incomplete knowledge of the key players, which are of crucial relevance in endometrial receptivity. In the present study a proteomics based approach was adopted to generate a catalogue of the major secreted and cellular proteins expressed during the proliferative and mid secretory phase/receptive in fertile women.
Methods: Women of reproductive age with a history of regular menses were enrolled in the study. Uterine fluid samples collected during the mid secretory phase (n=6) and proliferative phase (n=5) were resolved by two dimensional gel electrophoresis in the linear range of pI 4-7. Fifteen protein spots found differentially expressed were excised from the 2D gel of mid secretory phase uterine fluid and identified by MALDI-TOF. On comparing the mid secretory phase endometrial tissue with that of the proliferative phase 8 proteins were found differentially expressed between the two phases. Immunohistochemical analysis was performed to confirm the inferences drawn from the densitometric analysis of the 2D gels.
Results and discussion: Mass spectrometric analysis of the spots in the mid secretory phase uterine fluid revealed the identity of several proteins including alpha-1 anti-trypsin precursor, anti-chymotrypsin precursor, and β actin fragment. It is for the first time that the presence of HSP 27 was demonstrated in human uterine fluid. Our study is the also the first to report cycle dependent variations in the expression of endometrial calreticulin. The present study offers valuable clues regarding the differential expression of novel as well as some already known proteins expressed during the mid secretory/receptive phase of the endometrium. Some of these identified proteins could be analyzed further to be proposed as biomarkers to distinguish the receptive and non receptive state of the endometrium.

Notes

P9 Localisation of Inhibitor of Differentiation (ID) proteins in the sheep ovary

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Introduction: Inhibitor of Differentiation (ID) proteins, are negative regulators of basic helix loop helix (bHLH) transcription factors and function to repress transcription during vital windows of embryogenesis and development. Four *ID* genes (*ID1-4*) have previously been described and reported to regulate growth and differentiation, yet their function in adult physiology and pathology is less well determined. Here, we investigate a role for ID proteins in the adult ovary, a tissue persistently undergoing differentiation and remodelling.
Methods: ID1, ID2 and ID4 protein expression in the sheep ovary was examined *in vivo*, across different functional stages, by immunohistochemistry (n=14).
Results and discussion: ID1, ID2 and ID4 were consistently expressed in granulosa cells and to a lesser extent in the thecal layer of ovarian follicles throughout development. The ID1 protein was most strongly localised to granuolsa cells and exhibited striking peri-oocyte up-regulation in the cumulus layer, a feature observed in each oocyte examined. This may be indicative of a bidirectional oocyte-cumulus signalling pathway mediating developmental competence. ID2 was absent in atretic follicles revealed by positive activated caspase-3 expression and therefore may have a role in regulating cell death. ID proteins may also confer differential luteal functions since ID1 and ID4 were exclusively localised to large and small luteal cells, respectively, whereas ID2 was not expressed in the sheep corpus luteum. Finally, all three ID proteins were expressed in blood vessels, with the ID2 antibody exhibiting the most intense staining, and also in the ovarian surface epithelium, both tissues comprising cells that undergo constant physiological change to maintain ovarian function. These data collectively demonstrate a wide spectrum of regulated ID protein expression in the sheep ovary, suggesting important roles during follicle growth and development as well as atresia. ID regulation of gene transcription is likely ovarian wide with the evidence implying non-redundant as well as potentially overlapping functions.

P10 Influence of cycle stage and lactation on expression of Insulin-like Growth Factor Binding Proteins (IGFBPs) in the bovine oviduct

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Introduction: The oviduct provides the critical environment supporting fertilization and early embryo development. Early embryonic death causes major losses to the dairy industry, with a higher incidence in cows with poor energy balance. IGFs influence the rate of embryo cell division. This study compared expression of three IGFBPs between Holstein-Friesian dairy cows (n=20) and heifers (n=17) at three stages of the oestrous cycle.
Methods: Animals were synchronised using CIDRs, prostaglandin and GnRH as needed to reach three stages of development of the second follicle wave (A, newly selected dominant follicle in the luteal phase; B, preovulatory follicle prior to oestrus; C, luteinized preovulatory follicle, after GnRH). Cows (lactation number 3.75±0.4, mean±SD) were slaughtered 82±2.4 days after calving, when daily milk yield was 26.2±6.3 kg. Mean age of the nulliparous heifers was 1.67± 0.03 years. A section from each pair of ampullae was dissected and frozen in liquid nitrogen for RNA extraction. Transcript copy numbers for IGFBP-2, -3 and -6 were measured using real time PCR. Mixed model analysis determined the effects of side, time and status (cow/heifer).
Results and discussion: Circulating IGF-I was higher in heifers than cows (466 cf 132 ng/ml, $P<0.001$). Side with respect to the dominant follicle did not affect IGFBP expression. Concentrations of all three IGFBPs increased from the luteal to pre-ovulatory phases, with no further significant alteration post LH surge (IGFBP-2, $P<0.056$: IGFBP-3, $P<0.03$; IGFBP-6, $P<0.051$). IGFBP expression was consistently higher in heifers than cows (IGFBP-2, $P<0.053$; IGFBP-3, $P<0.003$). These IGFBPs are generally considered to inhibit IGF action, with both IGFBP-2 and -6 having a higher affinity for IGF-II than IGF-I. The results indicate tight regulation of IGF bioavailability in the oviductal environment around oestrus, with pronounced differences between cows and heifers. Future studies will assess the implications for embryo development.

Sponsored by Royal Thai Government and Science Foundation Ireland

P11 Induced hyperprolactinaemia promotes early luteal function in the female brushtail possum (Trichosurus vulpecula) **Notes**

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Introduction: The distinct pattern of prolactin (Prl) secretion during the peri-oestrus period in the brushtail possum suggests that Prl may play a role in luteal function in this species. To determine this, we induced hyperprolactinemia in female possums to assess effects on luteal function.
Methods: Lactating adult female possums had osmotic minipumps, loaded with either saline (control; n=8) or recombinant possum Prl (n=10), inserted (i.p.) under anaesthesia. Pouch young were removed (RPY) and the resumption of oestrus activity was monitored daily by vaginal cytology. Blood samples were collected every 1-2 days throughout the study via an indwelling jugular cannula. Animals were killed either 10 days after detection of oestrus and/or mating, or 23-25 days after RPY in those animals that failed to ovulate. Pituitary glands and corpora lutea (CL) were removed and frozen. Mean plasma Prl and progesterone (P_4) concentrations were measured by homologous and heterologous RIA, respectively. Mean expression levels of intra-pituitary *PRL, LHB, FSHR* and *GNRHR,* and CL-derived *LHR* mRNA were quantified by multiplex QPCR.
Results and discussion: In possums with induced hyperprolactineamia, 7/10 animals ovulated compared with 7/8 control animals. Plasma Prl concentrations in treated animals were not different to those in control animals, although the variability of concentrations was reduced suggesting a self-regulatory role for Prl. Plasma P_4 concentrations were higher ($P<0.05$) around the time of ovulation in treated animals although similar expression levels of *LHR* mRNA were observed in 10-day old CL. Mean intra-pituitary mRNA expression levels of *FSHB* were elevated ($P<0.05$) in treated possums, whilst *PRL, LHB,* and *GNRHR* were not different between groups. This study shows for the first time in a marsupial species that Prl may act through FSH to indirectly enhance P_4 production in luteinising granulosa cells at impending ovulation and during the early luteal stage in the brushtail possum.

P12 A comparison of in vitro prostanoid production in menorrhagic myometrium from women with and without uterine fibroids

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Introduction: The aim of this study was to simultaneously profile, using electrospray ionization liquid chromatography mass spectrometry (ESI/LC-MS/MS), prostanoids produced in samples of myometrium taken from non pregnant menorrhagic women with (n=5) and without (n=9) uterine fibroids.
Methods: This study was approved by the Bradford Teaching Hospital NHS Trust Ethical Committee, whereby samples obtained at hysterectomy from consenting women (mean age 46.2 years in fibroid group and 43.2 years in non-fibroid group) were transported to the laboratory and immediately placed in the -80ºC freezer to await subsequent solid phase extraction. Extracts analysed using ESI/LC-MS/MS were quantified using calibration lines of commercially available standards. Results were expressed as mean pg/mg protein (as estimated by Lowry method).
Results and discussion: Our data show simultaneous measurement of ten prostanoids in non pregnant myometrium with and without fibroids. PGD_2 predominated in the myometria of both groups, whilst 6-keto-$PGF_{1\alpha}$ (prostacyclin metabolite) was the second most abundant eicosanoid synthesised. PGD_2, TXB_2, 6-keto-$PGF_{1\alpha}$, PGD_1 and $PGF_{2\alpha}$ was significantly lower in myometria found to contain fibroids ($p>0.05$). 15-keto-PGE_2, PGE_2, $PGF_{1\alpha}$ and PGE_1 were present in reduced quantities in the fibroid group, although differences were not statistically significant. Low level production of PGJ_2 (a PGD_2 metabolite, 0.5 pg/mg protein) was observed for both groups. Menstrual phase, fibroid containing myometrial samples produced the highest concentrations of prostanoids compared to follicular and luteal phase samples. Myometrium from an endometriosis sufferer (n=1) also produced lesser quantities of prostanoids compared to myometrium from non endometriosis sufferers (n=9). The reduced concentrations of myometrial prostanoids found in women with uterine fibroids could explain why ibuprofen had no effect on blood loss in this group (Makarainen & Ylikorkala 1986) and why NSAID therapy was effective in reducing heavy menstrual bleeding in women without uterine fibroids.

Notes

P13 Characterization of GTSF1 expression during bovine and human oogenesis

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Introduction: Gametocyte Specific Factor 1 (*GTSF1*, also termed *FAM112B*) belongs to a functionally uncharacterized protein family with a conserved protein domain UPF0224. The gene is similar to the *Xenopus* oocyte specific D7 transcript which is involved in oocyte maturation. Using differential display and semi-quantitative PCR we have identified *GTSF1* as an oocyte specific marker during human ovarian folliculogenesis and have completed cDNA sequence analysis of *GTSF1* over 8 exons (exons 3-10). The objective of the present work was to characterise *GTSF1* expression during bovine and human oogenesis.
Methods: *GTSF1* gene expression was quantified by real-time PCR analysis relative to *Ywhaz* and *H2a* expression in immature and mature, metaphase II (MII), bovine oocytes from natural cycles and gonadotrophin-stimulated cycles. *GTSF1* was cloned from verified, staged SMART^{TM} amplified bovine and human oocyte cDNA libraries which were prepared as previously described (Huntriss et al., 2002; Mol. Hum. Reprod. 8: 1087). The cellular localisation of *GTSF1* expression was investigated in bovine ovary using fluorescent *in situ* hybridization (FISH) of paraformaldehyde fixed tissues.
Results and discussion: Semi-quantitative PCR showed oocyte specific expression *of GTSF1* during human follicle development. FISH localised *GTSF1* expression to the oocyte cytoplasm across all stages of bovine folliculogenesis from primordial stages onwards. Real-time PCR analysis confirmed *GTSF1* expression in human ovary and testis. High testicular expression suggested that *GTSF1* may have a primary function during spermatogenesis. In the bovine ovary, *GTSF1* expression was significantly higher in natural cycle MII (n=4) and immature (n=7) oocytes, irrespective of follicular maturity, than in MIIs oocytes (n=15) harvested from large follicles after controlled gonadotrophin stimulation. These data indicate that *GTSF1* is relevant to the regulation of oocyte development in monovular species. The precise function of *GTSF1* during mammalian oocyte maturation requires further clarification.

This work was supported by the BBSRC and MRC

P14 Molecular indices of oocyte quality in bovine follicles harvested from natural cycles and following controlled ovarian stimulation

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Introduction: During controlled ovarian stimulation (COS) the natural mechanism of follicle selection is overridden by exposure to excess, exogenous FSH. These regimens produce a hierarchy of follicles which maximise oocyte quantity at the expense of oocyte quality. We have previously demonstrated that oocyte *GDF9* expression following COS differed from expression in subordinate oocytes from natural cycles (NC). The present study investigated the utility of a panel of somatic and oocyte genes as markers of oocyte developmental competence.
Methods: Reproductive cycles of 12 heifers were down-regulated with Buserelin prior to ovarian stimulation by i.v. infusion of: (i) oFSH (50µg/h for 120h, n=6); or (ii) oFSH+oLH (oFSH 50µg/h for 72h followed by oLH 25µg/h for 72-120h. hCG (5000IU, i.m.) was injected at 120h and OPU performed 16-20h later. Real-time PCR was used to compare gene expression in granulosa cells, cumulus cells and oocytes from NC preovulatory (dominant) and subordinate follicles with cells derived following COS.
Results and discussion: Expression of *AMH, LHR, FSHR, FST, FGF8, EGFR, TSG6*, *PTGS2, PTX3, HAS2, GREM* and *SERP2*, were analysed in cumulus (n=28) and granulosa cells (n=16). Gene expression in granulosa cells from NC dominant follicles tended to exceed subordinates. *AMH* and *FST* expression were significantly higher ($P<0.05$) in cumulus cells from NC subordinate vs. COS follicles whereas *TSG6* and *PTGS2* were significantly lower ($P<0.05$). Expression of *BMPs -4, -6,* and *-15* tended to be higher in Metaphase II (MII) oocytes from NC dominant (n=5) and COS (n=8) follicles than NC subordinates (n=8). In contrast, *GDF9* and *GTSF1* expression were lower in COS MII oocytes compared to NC oocytes. These data demonstrate molecular differences between oocytes and somatic cells in follicles of different maturity within the ovulatory hierarchy. Further analysis will provide insight into the impact of COS on these markers of oocyte quality.

P15 Influence of leptin on steroidogenic secretion and nuclear in vitro maturation via MAPK pathway in the rabbit oocyte model **Notes**

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Leptin reflects the amount of body fat and could acts as modulator of oocyte quality during preovulatory follicular development through activation of specific transcription factors. The aim of this work was to study; 1) the influence of leptin on meiotic oocyte maturation and steroid secretion by cumulus-oocyte complexes (COC) and; 2) if mitogen-activated protein kinases (MAPK) are involved in leptin-associated signal transduction during *in vitro* maturation (IVM) of rabbit oocytes. A total of 773 COC (6 replicates) from follicles β1mm were *in vitro* matured for 16h (38ºC, 5% CO_2) in TCM199 plus: Exp.1) 10% foetal calf serum (FCS), 0 or 10ng/ml leptin; Exp.2) 0 and 10ng/ml leptin either supplemented with 10 and 50μM of the specific inhibitor of leptin-induced MAPK phosphorilation (Cell Signaling PD98059). Spent culture media were analyzed for oestradiol secretion by COC using EIA (Demeditec). To asses meiotic maturation (metaphase II rate), COC were incubated with 10μg/ml propidium iodide, and observed under a fluorescent microscope. Statistical analysis was performed by ANOVA and Chi-square tests. COC cultured with 10ng/ml leptin and FCS showed significantly higher nuclear maturation rates than those IVM with 0ng/ml leptin (75.2±2.3 and 71.6±5.6 *vs.* 54.1±9.6%, respectively, $P<0.005$). However, only COC of FCS group showed significantly higher oestradiol secretion compared to 0 and 10ng/ml leptin treatments (4.1±0.7 *vs.* 0.1±0.03 and 0.3±0.1pg/COC, respectively, $P<0.001$). Leptin-stimulated oocyte maturation was significantly impaired when leptin-induced MAPK phosphorilation was suppressed by the MAPK inhibitor at both 10 and 50μM concentrations (75.2±2.3 *vs.* 44.1±5.5 and 49.3±9.1%, respectively, $P<0.001$). The addition of MAPK inhibitor without leptin supplementation did not reduce the MII rate (54.1±9.6 *vs.* 51.5±6.6 and 54.4±5.0, respectively). The results confirm that leptin enhances meiotic oocyte maturation through activation of MAPK pathway in rabbit oocyte model, but oestradiol secretion was not influenced.

We acknowledge CM, FSE and MICINN for funding.

P16 Expression of hyaluronan synthases in the ovine cervix varies with the stage of oestrous cycle

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Introduction: In the ewe there is a degree of natural relaxation of the cervix at oestrus, which may be initiated by binding of Hyaluronan (HA) to its receptor CD44. Previously we have shown that HA content of the ovine cervix and fragment size of HA varies with the stage of the oestrous cycle. Hyaluronan syanthases (HAS) are membrane proteins that regulate HA biosynthesis. This study determines whether HAS1, 2, and 3 are expressed in the ovine cervix, and if so, how their expression alters during the oestrous cycle.
Methods: Cervices were collected from 24 cyclic sheep (n = 8 / group) at the luteal, pre-LH and post-LH surge stages. Protein and mRNA expression for HAS1, 2 and 3 was determined in 5 different tissue layers (epithelium, sub-epithelial stroma, and longitudinal, circular and transverse muscle) of the vaginal, mid and uterine regions of each cervix by immunohistochemistry and *in situ* hybridisation, respectively. Expression for HA synthases was semi-quantitatively assessed in each layer of each region using percentage of cells showing positive staining along with intensity of staining. Data were analysed by ANOVA.
Results and discussion: HA synthases were expressed in all the tissue layers and regions of the cervix, and the pattern of expression was similar for mRNA and protein. HAS1 protein expression was significantly ($P\leq0.05$) higher in the pre-LH surge stage, whilst HAS2 and 3 protein expression was significantly ($P\leq0.001$) higher in the luteal stage. HAS expression was significantly ($P\leq0.001$) higher in the epithelial layer and the vaginal region. These findings are in accordance with our previous results and explain the differences observed in the HA content, differing HA fragment size and CD44 expression at different stages of the oestrous cycle. These data suggest that progesterone along with oestradiol and LH may be implicated in the regulation of cervical HA synthases.

Notes

P17 Prostaglandin synthesis in luteinizing bovine granulosa cells

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Prostaglandins (PGs) exert regulatory actions during different stages of the oestrous cycle. PGs are generated by the sequential metabolism of arachidonic acid by cyclooxygenase enzymes (COX-1 - constitutionally active, COX-2 - inducible) and terminal prostaglandin specific synthases to yield bioactive 2-series PGs. Although PGs are essential for ovulation and luteolysis, their role during luteinisation remains unelucidated.

Cells from antral follicles (2-8mm) were isolated and cultured for 8 days (d) (with media being changed every other day) at a density of 3 x 10^6/ml. Spent culture media was assessed for prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and E_2 (PGE_2), progesterone (P_4) and oestradiol-17β (E_2) by radioimmunoassay. COX-1 and COX-2 gene expression was assessed by PCR. Measurements are expressed as a percentage of d1 (100%).

During luteinisation, $PGF_{2\alpha}$ declined rapidly (d4; 41%, d8; 51%) which was coincident with a decrease in PGE_2 (d4; 27%, d8; 59%). These changes were associated with a decrease in COX-2 gene expression on d5 (d5; 15%, d8; 49%), while levels of COX-1 increased (d4 325%) and declining to 44% by d8. It is interesting to note that levels of PGE_2 and COX-2 declined coincidently whereas the decrease in $PGF_{2\alpha}$ preceded that of COX-2. As expected, levels of E_2 declined over the culture period (d4; 75%, d8; 78%) whereas P_4 levels continued to increase (d4; 189%, d8; 371%).

Data presented here provides support for the notion that the inducible terminal enzyme for PGE_2 synthesis, mPGES1, is coupled to COX-2. The observed decrease in levels of $PGF_{2\alpha}$ which preceded that of COX-2 could potentially be explained by invoking a decrease in $PGF_{2\alpha}$ synthase (PGFS). The inability of COX-1 to maintain $PGF_{2\alpha}$ levels despite a substantial increase in COX-1 expression provides further support for this contention. The dynamic changes of PGs observed here demonstrate a potential regulatory role in luteinisation.

P18 Epigenetic reprogramming during wound repair

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Introduction: Wound healing is a dramatic regenerative episode that requires epithelial cells to proliferate, migrate, and differentiate, and this in turn requires induction of a vast repair transcriptome, including cell cycle regulators, matrix molecules, integrins, proteases, antioxidant enzymes, etc. Epigenetic modifications have recently emerged as key regulators of gene transcription potential, and we hypothesized that epigenetic reprogramming, and specifically changes in histone and DNA methylation, may allow for un-silencing of repair genes. Polycomb group proteins (PcGs) Ezh2, Eed, and Suz12 co-operatively repress large numbers of target genes by laying the repressive mark histone H3 lysine 27 tri-methylation (H3K27me3), which can be removed by histone demethylases Utx and Jmjd3.

Methods: To determine whether PcGs and their demethylases contribute to regulating gene expression during repair, we analyzed mouse skin wounds for changes in their expression and activity.

Results and discussion: Eed, Ezh2, and Suz12 were significantly down-regulated in wound-edge epithelial cells, whereas Jmjd3 and Utx1 were up-regulated. Moreover, we found a striking reduction of the repressive mark H3K27me3. Data mining of array experiments identifying potential PcG target genes indicates that up to 20% of known repair genes may be regulated by the loss of polycomb-mediated silencing. Quantitative chromatin immunoprecipitation studies revealed that indeed there is less Eed bound to the regulatory regions of two paradigm wound-induced genes, Myc and Egfr, during repair.

This study reveals transient epigenetic changes in an acute wound model, but we speculate that in repetitive or chronic wounds, such as ovulatory wounds, these changes in polycomb expression may fail to resolve, resulting in prolonged, and potentially tumorigenic deregulation of their targets. This may provide a molecular explanation for the increased susceptibility to tumour formation at chronic wound sites, including the ovary. I am presently extending my research to encompass the cell biology and regulation of gene expression during ovulatory wound repair.

P19 Dysregulation of ovarian 11B-hydroxysteroid dehydrogenase (11BHSD) activity in anovulatory polycystic ovary syndrome (PCOS) **Notes**

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Introduction: Within potential target cells, 11BHSD enzymes catalyse the oxidative inactivation of cortisol. Since altered hepatic cortisol-cortisone metabolism by 11BHSD1 has been linked with PCOS, we aimed to establish whether ovarian 11BHSD activities are altered in PCOS and to assess the possible effect of insulin and androgens on ovarian cortisol metabolism.
Methods: Cortisol and cortisone concentrations were measured (by specific RIAs) in follicular fluid (FF) aspirated from size-matched follicles dissected intact from 14 normal, 24 ovulatory polycystic ovaries (PCO) and 13 anovulatory PCO. Human granulosa cells, recovered during oocyte retrieval for IVF, were cultured in 1:1 DMEM:Ham's F12 medium supplemented with 10% (v/v) foetal calf serum. On day 4 in culture, cells were washed into serum-free medium and 11BHSD1 activities were measured in the presence of insulin (100nM) ± metformin (1μM) or a range of androgens/oxy-androgen metabolites (0.01-10μM). Following 4 hour incubation with 100nM ^{3}H-cortisol, ^{3}H-steroids were extracted into chloroform, resolved by thin layer chromatography and quantified using a radiochromatogramme scanner.
Results and discussion: Intra-follicular cortisol:cortisone ratios were elevated in FF from anovulatory PCO (2.1±0.4; $P<0.05$) but did not differ between ovulatory PCO (1.6±0.1) and normal ovaries (1.2±0.1). FF concentrations of cortisol and cortisone and the follicular cortisol:cortisone ratios did not correlate with follicular diameter (R^2=0.030, 0.005 and 0.000, respectively). 11BHSD1 activities assessed in vitro were lower in IVF patients with PCOS than in women with all other causes of infertility (median = 5.8 vs 14.9mol cortisone/4h, respectively; $P<0.05$). Cortisol oxidation was unaffected by insulin ± metformin, but was acutely inhibited in a concentration-dependent manner by testosterone, 11B-hydroxyandrostenedione, 7-alpha-hydroxy- and 7-beta-hydroxy-DHEA ($P<0.01$). Hence, there is decreased inactivation of cortisol in follicles from anovulatory PCOS. This may reflect inhibition of 11BHSD1 by androgens and their 7/11-oxy-metabolites, local concentrations of which are increased in PCOS.
Funded by a grant from the University of London Central Research Fund.

P20 Use of pathway-focused QPCR arrays for profiling changes in ovarian expression of transforming growth factor-β (TGFβ) system components in FSH receptor 'knock-out' mice

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Introduction: Ovarian follicle development is regulated through both endocrine (i.e. FSH, LH) and intraovarian control mechanisms. Mounting evidence supports a role for intraovarian TGFβ superfamily members including inhibins, activins, bone morphogenetic proteins (BMP) and growth & differentiation factors (GDF). In this study we examined in adult mice the impact of targeted deletion of the gene encoding FSH receptor (FSHRKO) on ovarian morphology and on expression of a panel of over 80 genes associated with TGFβ superfamily signalling.
Methods: Ovaries from adult (10-12 week-old) control and FSHRKO mice (6-8 per group) were weighed and either snap-frozen for RNA extraction and cDNA synthesis or fixed in Buoin's for histology. cDNA samples were applied to TGFβ superfamily pathway-focused QPCR arrays (SABiosciences, MD, USA). Array processing and data analysis were done according to manufacturers instructions. The ΔΔCt method was used for relative quantitation of transcript abundance in control (4 arrays) versus FSHRKO mice (3 arrays).
Results and discussion: Ovaries of FSHRKO mice were smaller than controls ($P<0.01$) and lacked antral follicles and corpora lutea although all other follicle stages were represented. Of 83 targeted transcripts on the array, 23 (28%) were significantly ($P<0.05$) down-regulated by >2-fold in FSHRKO versus controls while only 3 (4%) of transcripts (chordin, IGF1, LTBP2) were upregulated (>3-fold; $P<0.01$). Down-regulated transcripts included various TGFβ family ligands (BMP2 -3, -6, -7, GDF3, -5, -6, -7, inhibin/activin βA and βB), binding proteins (follistatin, noggin, LTBP1) and receptors (ActR2A, TGFβR1), as well as the cell-cycle regulators CDKn1a and CDKn2b. These observations indicate the utility of pathway-focused QPCR arrays for rapid expression profiling of selected signalling pathways. While the marked reduction in inhibin/activin βA/βB subunit expression in FSHRKO mice confirms previous work, this study has revealed over 20 other 'novel' TGFβ-pathway components whose expression is associated with FSH-dependent follicle progression beyond the preantral stage.

Notes

P21 Oocytes lacking core 1-derived O-glycans increase female fertility by modifying folliculogenesis in mice

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Introduction: The number of eggs ovulated is regulated in a species-specific manner and despite considerable research, the regulatory mechanisms remain unclear. To investigate the mechanisms that regulate how many ovulatory follicles are generated, we are using a novel mouse model with sustained increased fertility. The *T-syn* mutant generates oocytes lacking core 1-derived O-glycans by oocyte-specific deletion of the T-synthase enzyme. Folliculogenesis was investigated in *T-syn* mutants to determine how the lack of oocyte-generated O-glycans leads to increased fertility.
Methods: Females homozygous for a floxed *T-syn* gene and carrying a *Cre* recombinase transgene under the control of the oocyte-specific ZP3 promoter generate oocytes lacking core 1-derived O-glycans. Folliculogenesis was examined in pre- and postpubertal mice.
Results and discussion: The increase in litter size of *T-syn* mutants is due to an increase in the number of eggs ovulated. Mutant ovaries are heavier and contain increased numbers of follicles specifically at the preantral and antral stages of development. Furthermore, follicles classed as preantral or antral were larger in the mutant compared to controls. Mutant follicle numbers were not increased due to a decrease in the number of atretic follicles, however the number of apoptotic cells in atretic mutant follicles was reduced compared to atretic control follicles. These data support a model of slowed follicle development at the preantral and antral stage. We hypothesise that follicles continue growing at the gonadotrophin-independent preantral stage for longer prior to advancing to the gonadotrophin-dependent antral stage. A decrease in the superovulation rate of prepubertal mutant females compared to controls despite increased numbers of follicles supports this hypothesis. These data clearly demonstrate a role(s) for oocyte glycoproteins in the regulation of follicle development and subsequent ovulation rate. Our future studies aim to elucidate the mechanisms involved to further our understanding of how ovulation rate is regulated.

P22 Alteration of placental cortisol metabolism in human post-term labour

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Introduction: During pregnancy, the placenta maintains a balance between the physiological glucocorticoid cortisol and its inactive metabolite, cortisone. Two 11B-hydroxysteroid dehydrogenase (11BHSD) isoenzymes catalyse this reaction. 11BHSD1 is a NADP(H)-dependent, bidirectional enzyme whereas 11BHSD2 acts solely as a NAD^+-dependent dehydrogenase. In pre-term labour, local cortisol levels are increased in the placenta following a decrease in the expression and activity of 11BHSD2 (relative to normal term delivery). We reasoned that if the decrease in placental 11BHSD2 plays a causal role in triggering parturition, this enzyme activity would be increased in post-term labour. The aim of this study was to assess whether placental 11BHSD activities in post-term deliveries differed from normal term labour.
Methods: Placental samples were obtained following spontaneous vaginal deliveries at term (38-40 weeks). Post-term samples (at 42 weeks of gestation) included spontaneous or induced onset of labour with vaginal delivery or Caesarean section. Placental tissues were homogenised (blinded to whether samples were term/post-term) and 11BHSD activities measured by incubating tissue for 10mins at 37^{o}C with 100nM [^{3}H]-cortisol plus 0.4mM $NADP^+$ or NAD^+. Steroids were extracted, resolved by TLC and quantified using a radiochromatogramme scanner. (These studies were approved by Wandsworth Regional Ethics Committee).
Results and Discussion: Initial assays confirmed that the major placental enzyme activity was NAD^+-dependent and that both 11BHSD activities were higher in the placental periphery than in central regions. In tissue homogenates prepared from the placental edges, rates of NAD^+-dependent cortisol oxidation tended to be lower in 10 post-term placentas than in 19 term deliveries (4.9±0.8pmol vs. 8.2±1.5pmol cortisone/mg protein.10min; P=0.063). Since post-term delivery is not associated with a significant increase in the placental inactivation of cortisol, the decreased placental 11BHSD2 activity previously associated with pre-term labour may not play a causal role in timing parturition in women. This work was supported by the St. George's Charitable Trust.

P23 The influence of age on genotoxic effects of ENU on mouse spermatogenic cells **Notes**

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Introduction: Recent findings concerning the age-related increase in the mutation rate in male germ cells have demonstrated that a number of mechanisms are likely to contribute. These include differential responses of aged and young testes to genetic damage and survival advantages conferred by certain mutations in spermatogonia. As part of an on-going series of studies into this phenomenon, we have investigated in mice how the ability of ethylnitrosourea (ENU) to damage germ cell DNA varies with age.
Methods: Groups of 10 male, 102EIxC3HEI mice aged either 2 months (young adult) or 17 months old (>80% of lifespan), were treated with a single dose of ENU (250mg/kg bwt) or vehicle and examined either 24 hours later to examine the susceptibility of mature sperm or 8 weeks after (ENU) to examine spermatogonia. The young and old control groups from these two studies (24-hour time point only) were used to examine the effects of age alone. Testes weight and sperm counts were recorded and the Sperm Chromatin Structure Assay (SCSA) performed. Apoptosis among testicular germ cells was assessed by the Terminal deoxyUridine Nick End-Labelling (TUNEL) assay and point mutations in spermatogonia are being examined by inverse Restriction Site Mutation (iRSM) assay.
Results and discussion: ENU caused a wide range of effects on the end-points examined and effects of age were also apparent. Interestingly, although testes of aged mice were lower than young, the sperm counts were unchanged. No effect was seen with the SCSA 24 hours after dosing but 8 weeks later the effect was marked. TUNEL staining revealed a profound induction of apoptosis by ENU and comparisons between young and aged animals yield insights into the mechanisms by which some of the other effects may be caused.

P24 Metal induced oxidative stress and altered testicular steroidogenesis and spermatogenesis and the protective role of melatonin

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Introduction: Testicular toxicity to a trimetallic combination(Ni, Cd & Cr(VI)) has been assessed in an environmentally relevant realistic dosage schedule. The study had been undertaken as humans are exposed to multiple metals in industrialized areas and Ni, Cd & Cr(VI) are the predominant metals in the environs of Vadodara.
Methods: Adult *wistar* rats were exposed to Ni(40mg/Kg BW), Cd(9mg/Kg BW) and Cr(VI)(20mg/kg BW) through drinking water with or without simultaneous administration(*i.p.*) of melatonin(10mg/Kg BW) at 1800hrs for 60 days. All animals were maintained as per the guidelines of CPCSEA, India. On 61st day, 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) alongwith TBARS and activity levels of steroid dehydrogenases and testicular NO level by using appropriate assay procedure/ kit. Histopathology of testis, epididymal sperm analysis and assay of serum titres T, E_2 & Melatonin were also carried out.
Results and discussion: Metal exposure induced significant increased in lipid peroxidation and decreased in antioxidant levels together with decreased activity of steroidogenic enzymes and lowered serum T and E_2 levels. Significant, increased in testicular metal load(Cr>Cd>Ni) and reduced serum melatonin level were also observed. Increased testicular NO level with disruption in spermatogenesis and severe sperm abnormality were the other features of relevance. Co-administration of melatonin afforded significant protection against all the above trimetallic toxicity manifestations. Results from the present study suggest that male reproductive toxicity due to long term exposure to metals manifest essentially due to increased oxidative stress and disrupted steroidogenesis resulting in lowered serum steroid hormone profile leading to disruption in spermatogenesis and sperm abnormalities and that, melatonin could be an effective counter-protective agent.

Notes

P25 Characterization of immortalized steroid-responsive pig endometrial cell lines

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Introduction: Epithelial and stroma cells of the endometrium is the site were information from embryo is gathered and generated to maternal system. Creation of stable *in vitro* endometrial model may be helpful to study complex signaling pathways during maternal recognition of pregnancy and implantation. Recently we have immortalized endometrial epithelial and stroma cells from Day 3 of oestrous cycle. These cells maintain morphology and specific markers of the cells which were isolated from. Both cell lines expressed PR and ERs receptors. The study aimed further characteristic of obtained endometrial cell lines as a next step to develop a three dimensional model of pig endometrium.
Methods: Cells were cultured in complete M-199 medium. Before experiment cells were incubated in steroid free medium overnight. Then, cells were exposed to P4 (10, 100, 1000 nM) and E2 (1, 10, 100 nM). After 24 h, culture medium was collected for EIA of PGs and cells for Western blot of COX-2 expression. A preliminary karyotype analysis from endometrial cell lines was made with Giemsa staining.
Results and discussion: Examined metaphases (20 at least from each line) revealed a typical porcine chromosome diploid number (2n=38). No large rearrangements in chromosomes were observed. COX-2 expression in stroma cells increased after 1 μM of P4 and 100 nM of E2 stimulation. Treatment of stroma cells with P4 (1 μM) and E2 (10-100 nM) resulted in significantly higher $PGF_{2\alpha}$ and PGE_2 secretion. Epithelial cells responded only to P4 stimulation with increased $PGF_{2\alpha}$ and PGE_2 release. In conclusion, immortalized endometrial cell lines appear similar physiology behavior to primary culture cells which makes them an ideal model for studying communication signals between embryo and uterus in pig.

Supported by MSHE research grant Nr NN311136033. J. Kiewisz, partially supported by the President of Polish Academy of Sciences (Nr 4/S/2007).

P26 Quantitative proteomic analysis of inter-caruncle sheep endometrium during peri-implantation period

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Introduction: Embryo implantation is a complex process involving a cross-talk between the endometrium and embryo. Better understanding of the molecular signals involved in implantation may lead to enhance implantation rate and improve pregnancy rates. We, therefore, employed proteomic techniques to study proteins alterations of sheep inter-caruncle during peri-implantation periods.
Material and methods: Inter-caruncle tissue of cyclic ewes assigned as controls and pregnant ewes were harvested on days 12 and 16 of oestrous cycle and on days 12, 16 and 20 of pregnancy, respectively (n=4 ewes/group). Equal amounts of protein from each ewe were pooled according to pregnancy and time point. Quadruplicate 11 cm, pH 3-10, 2-D gels were electrophoresed, Coomassie blue stained and analysed by Progenesis SameSpots software. 20 spots showing statistically significant changes in normalised spot volume ($p<0.05$) were identified using LC-MS/MS. Western blot was used to investigate proteins showing significant alterations between groups.
Results and discussion: The greatest number of significant spot alterations (37 spots up-regulated vs 15 down-regulated, $p<0.05$) occurred on comparison between cyclic ewes (C12 Vs C16), presumably because the CL began to regress thus implantation has not taken place. Whereas, the smallest number of significant spot changes was observed in pregnant groups between 12 and 16 days (6 spots up-regulated vs 25 down-regulated, $p<0.05$). Furthermore, 13 normalized spot volumes were down-regulated while 26 ones were up-regulated during the implantation compared to post-implantation periods in pregnant group (P16 vs P20). The day 12 non-pregnant inter-caruncle differed significantly from the pregnant inter-caruncle at the same time (36 spots down-regulated vs 8 ones up-regulated, $p<0.05$) and comparison between non-pregnant and pregnant inter-caruncle at the time of implantation demonstrated 17 spots down-regulated vs 17 spots up-regulated ($p<0.05$). These data and identified proteins may help to better understanding of the mechanisms involved in implantation and therefore improved rate of implantation and ongoing pregnancy.

P27 Expression and localisation of estrogen and progesterone receptor mRNA and protein in the bovine endometrium: the effect of pregnancy and progesterone supplementation **Notes**

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Introduction: Estrogen and progesterone (P4) mediate the reproductive process through binding their nuclear receptors (ESR and PGR respectively) in the uterus. This study aimed to determine the effect of P4 supplementation and pregnancy on gene expression and protein localisation of these steroid receptors.
Methods: Beef cross heifers (N=263) were synchronized and randomly assigned to one of four groups: (i) cyclic, normal P4 (ii) cyclic, high P4 (iii) pregnant, normal P4 (iv) pregnant, high P4. The latter group has been associated with advanced conceptus elongation. Elevated P4 was achieved through PRID insertion on Day 3 resulting in significantly elevated P4 concentrations from Day 3.5 to 8 ($p<0.05$) in the high P4 groups compared to the controls. Uterine tissue was collected on Day 5, 7, 13 or 16 of pregnancy/estrous cycle. ESR (,) and PGR mRNA expression was determined using Q-RT-PCR and protein localisation was carried out by immunohistochemistry
Results and discussion: *PGR* and *ESRα* expression decreased between Days 7 and 13 in all treatments ($p<0.05$) and was not affected by elevated P4. *ESRβ* expression was highest on Day 7 ($p<0.05$). PGR protein was localised in the endometrium (luminal epithelium, stroma, superficial and deep glands) and myometrium in all treatments in Days 5 and 7. However, by Day 13 and 16, there was either no or a very weak signal in the luminal epithelium and superficial glands. Intensity within deep glands, myometrium and stroma remained detectable from Day 5 to 16. P4 supplementation advanced disappearance of PGR protein from the luminal epithelium and superficial glands in Days 5 and 7 compared to the controls. ESR protein was localised throughout the endometrium and myometrium in all observed days. In conclusion, pregnancy did not affect expression of either *ESR* or *PGR* genes or protein but progesterone supplementation suppressed PGR protein expression. ESR expression was not regulated by P4.

P28 Glutathione supplementation of the extender improves the quality of cryopreserved buffalo (*Bubalus bubalis*) bull semen

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Artificial insemination with frozen-thawed semen is not quite successful in buffalo and results in poor fertility. Cryopreservation lowers the antioxidant levels and puts spermatozoa under oxidative stress. The objective of this study was to determine whether glutathione (antioxidant) supplementation of the extender improves the quality of cryopreserved buffalo spermatozoa.
Semen from 5 buffalo bulls was diluted at 37°C with tris-egg yolk extender (50×10^6 motile spermatozoa ml^{-1}) supplemented with 0.0, 0.5, 1.0, 2.0 or 3.0mM glutathione. Diluted semen was cooled to 4°C in 2h and equilibrated for 4h at 4°C. Semen was then filled in 0.5 ml French straws and kept on liquid nitrogen vapours for 10 min. Straws were then plunged and stored in liquid nitrogen (-180°C). Assessment of semen quality was done at 0, 3 and 6h after thawing at 37°C for 30 seconds. Data were analyzed by ANOVA and Post-hoc comparisons for treatment and time were performed using LSD.
Pre-freezing glutathione supplementation of the extender upto 2.0 mM concentrations significantly ($P \leq 0.05$) increased sperm motility, sperm viability, plasma membrane integrity and acrosome integrity at 0, 3 and 6h after thawing in a dose-dependant manner compared to the controls. Glutathione supplementation at higher concentration (3.0 mM), however, did not prove to be beneficial to any of semen quality parameters and the values were not different from those of controls. There was a gradual decrease ($P \leq 0.05$) in semen quality with time after thawing. Morphological abnormalities of the sperm head, mid piece and tail did not differ among different concentrations of glutathione. The data obtained suggest that glutathione supplementation of tris-egg yolk extender upto 2.0mM improves the post-thaw quality of cryopreserved buffalo bull spermatozoa.

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