Society for Reproduction and Fertility Conference 2006 and National Ovarian Workshop

University of Leeds

3-5 July 2006

Programme & Abstract Book

Society for Reproduction and Fertility Abstract Series No. 33

ISSN 1476-3990

July 2006

www.srf-reproduction.org

WELCOME

On behalf of everyone involved in organising the SRF and NOW conferences for 2006, it gives us great pleasure to welcome delegates to the University of Leeds.

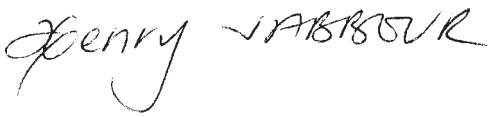
This year, the annual conference of the Society for Reproduction and Fertility is held in conjunction with the National Ovarian Workshop over three days. While the format of NOW will change slightly to fit into a single day, the format of the SRF annual conference remains as that followed in recent years. This three day joint meeting is packed with high calibre international and national speakers as well as Society prize sessions.

We have also organised a relaxed social programme that will culminate in our traditional SRF Conference dinner on Tuesday night.

We would like to thank Debbie Walker, Suzanne Ellingham and their colleagues at World Event Management (WEM) who are acting as Secretariat for this conference. The Staff at WEM have been very helpful and efficient in organising this joint conference, so please do not hesitate to contact them at the registration desk if there is anything that they can help you with during your stay in Leeds.

We hope that this joint meeting will provide delegates with opportunities both to catch up with old friends and colleagues and to establish new links. We also hope you will enjoy your visit to Leeds and wish you an enjoyable and productive conference.

Best wishes



Dr Henry N Jabbour

Dr Tony Michael

GENERAL INFORMATION

VENUE

The conference will take place at the University of Leeds, which is situated approximately ten minutes by foot from the heart of Leeds city centre.

One of the largest universities in the world, the University of Leeds is acclaimed world-wide for the quality of its teaching and research. Its broad research and skills base and superb facilities attract interest from major multinational and small local businesses alike.

The university has excellent meeting facilities including a large lecture theatre, a conference auditorium and concert hall. In addition there are several bars/cafés, an ATM machine and supermarket.

ACCOMMODATION

Accommodation has been arranged at the Merrion Hotel which is a ten minute walk from the University of Leeds. Special rates have been negotiated for SRF delegates. Please be advised that payment is to be made direct to the hotel upon checkout.

CAR PARKING

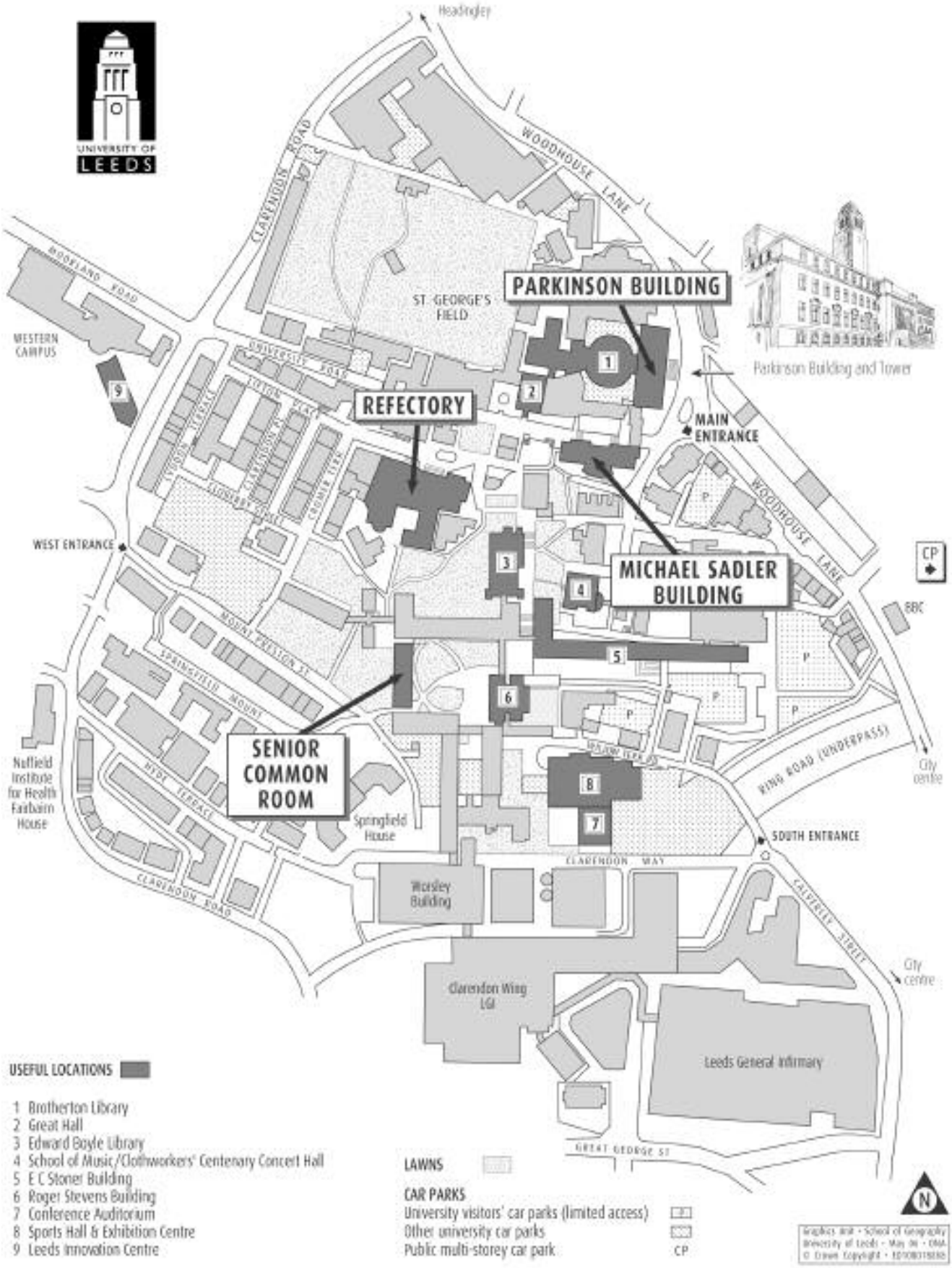
Car parking within the campus is very limited. As such delegates are advised to park their cars at the Merrion hotel. Parking is available in the adjacent Q Park with a walkway through into the hotel. Between 16.30 and 09.30 a reduced rate of £3.00 applies. Out of these times the standard fees apply. For more information please visit www.q-park.co.uk

LOCATION: LEEDS

Situated at the heart of the UK with a close proximity to the beautiful Yorkshire Dales, Leeds offers many attractions that bring over 11 million visitors to Leeds every year. Rich local history, world-class sports, fantastic shopping and diverse year-round entertainment are just some of the things that make Leeds such a great place for everyone to enjoy.

Leeds boasts a rich creative heritage. It hosts the Leeds International Film Festival, and is home to the West Yorkshire Playhouse. It is also the only English city outside London to have its own opera and ballet companies – the internationally acclaimed Opera North and Northern Ballet.

THE UNIVERSITY OF LEEDS CAMPUS MAP



MEETING INFORMATION

REGISTRATION

The registration desk will be situated in front of the Rupert Beckett Lecture Theatre and will be open at the following times:

- Monday 3rd July: 09.30 until 21.00
- Tuesday 4th July: 08.00 until 18.00
- Wednesday 5th July 08.00 until 16.00

Delegates will be able to collect their delegate packs from the registration desk at the above times.

Name Badge

Please ensure your name badge is worn at all times for security purposes.

Trade Exhibition

A number of trade exhibition stands are located in the Parkinson Court throughout the duration of the conference.

Poster Sessions

Poster submissions will be on display within the Parkinson Court throughout the conference. There will be an attended poster viewing session on Wednesday 5th July at 10.00 until 11.30 where coffee and tea will be served.

Catering

Tea and coffee will be available during the scheduled breaks throughout the meeting and will be served in the Parkinson Court. Lunch will also be served in this area.

SOCIAL PROGRAMME

Monday 3th July – Welcome Reception

There will be a complimentary drinks reception served in Parkinson Court of the University of Leeds from 18.00 – 19.00 amongst the trade stands and posters.

We invite all delegates to attend this reception to meet with colleagues and enjoy a glass of wine.

Following the drinks reception a Postgradoc quiz is organised in the Senior Common Room.

Tuesday 4th July – Conference Dinner

Dress Code: *Smart Casual*

A conference dinner is organised in the Refectory at Leeds University at 19.30.

We have live entertainment available whilst you enjoy a dinner and meet your colleagues.

Tickets have been pre-booked for this event but may also be purchased from the registration desk. Please ensure you bring your ticket to the event as proof of purchase.

EXHIBITORS

Ferring Pharmaceuticals

As a leading pharmaceutical company in the field of reproductive health, Ferring Pharmaceuticals offer high quality products for the treatment of infertility.

Menopur®, Ferring's highly purified menotrophin, is a highly purified gonadotrophin used for the treatment of infertility.

Menopur® provides a 1:1 ratio of FSH and LH activity and can be administered by subcutaneous injection.

At Ferring, we believe that patient information and support are essential and offer a comprehensive range of services designed to help make treatment easier.

QIAGEN

QIAGEN is the leading provider of innovative technologies and products for preanalytical sample preparation and molecular diagnostics solutions.

We have developed a comprehensive portfolio of more than 500 proprietary, consumable products and automated solutions for sample collection, and nucleic acid and protein handling, separation, and purification. We also supply diagnostic kits, tests, and assays for human and veterinary molecular diagnostics.

Our products are sold to academic research markets, and to leading pharmaceutical and biotechnology companies as well as to diagnostics laboratories. QIAGEN also provides purification and testing solutions to applied testing markets such as forensics, animal or food testing, and pharmaceutical process control.

We employ more than 1500 people worldwide. QIAGEN products are sold through a dedicated sales force and a global network of distributors in more than 40 countries. QIAGEN's Customer Satisfaction department focuses on continually exceeding our customers' increasing expectations. Our close contact with customers keeps us on the cutting edge of new technologies and identifies market needs that feed into our product development engine.

Reproduction

Reproduction aims to be the journal of choice for authors publishing high-quality research in the field of reproductive biology. It focuses on cellular and molecular mechanisms of reproduction, including the development of gametes and early embryos, assisted reproductive technologies in model systems and in a clinical environment, reproductive endocrinology and reproductive physiology.

- Online with HighWire Press
- The latest **Research** and topical **Reviews** in reproductive biology
- Easy online manuscript submission and peer-review
- **Review Articles** FREE to all immediately upon publication
- **Research Papers** free to all one year after publication
- FREE COLOUR AND NO PAGE CHARGES
- **Focus issues** in 2006 on 'TGF-beta signalling' and 'Stem cells in reproductive science'

The URL is www.reproduction-online.org

Visit the *Reproduction* stand to see an online demonstration of the journal or to take away your free sample copy of the journal, free postcard or poster.

Reproduction is published by BioScientifica Ltd, on behalf of the Society for Reproduction and Fertility, a not-for-profit society supporting research.

PROGRAMME

MONDAY 3RD JULY

09:30 – 10:30	*Registration*	*Foyer of Rupert Beckett Lecture Theatre, Michael Sadler Building*
10:30 – 11:30	**NOW Session 1** Comparison of the bioactivities and follistatin-binding properties of activin-A,activin-AB and activin-B **Claire Glister** **NO1** Purified follistatin isoforms from bovine follicular fluid (bFF) differ in their ability to bind and neutralise activin-A and associate with cell-surface proteoglycans **Claire Glister** **NO2** Activin Promotes *in vitro* Preantral Follicle Growth **Marie McLaughlin** **NO3** Bone morphogenetic protein-6 (BMP-6) greatly enhances gonadotrophin-dependent expression of mRNAs encoding the inhibin/activin subunits and gonadotrophinreceptors in granulosa cells of preovulatory (F1-F3/4) chicken follicles **Sara Al-Musawi** **NO4**	*Rupert Beckett Lecture Theatre, Michael Sadler Building*
11:30 – 12:15	*Coffee Break*	*Parkinson Court*
12:15 – 13:00	**NOW Session 2** 11βHSD activities in porcine oocytes and cumulus-oocyte complexes **Rachel Webb** **NO5** Ablation of Core 1 O-Glycan Synthesis in the Primordial Oocyte Enhances Female Fertility **Suzannah Williams** **NO6** The pro-apoptotic protein PDCD4 is a potential target for Dazl in mouse oocytes **Yvonne Brown** **NO7**	*Rupert Beckett Lecture Theatre, Michael Sadler Building*

PROGRAMME

13:00 – 14:15	*Lunch*	*Parkinson Court*
14:15 – 15:15	**NOW Session 3** *In Vitro* maturation of sheep cumulus oocyte complexes using a serum-free maturation medium **Matthew Cotterill** **NO8** Bovine egg culture and vitrification using V1/V2a media **Vanessa Moreira** **NO9** Effects of low physiological temperatures on pig oocyte nuclear maturation and kinase activity **Joanne Coleman** **NO10** APCcdh1 activity maintains germinal vesicle stage arrest of mouse oocytes **Alex Reis** **NO11**	*Rupert Beckett Lecture Theatre, Michael Sadler Building*
15:15 – 16:15	*Tea Break*	*Parkinson Court*
16:15 – 17:00	**NOW Session 4** Characteristics of follicular waves and associated ovarian events during the oestrous cycle in Nili-Ravi buffaloes undergoing spontaneous and $PGF_{2\alpha}$ induced luteolysis **Hassan Mahmood Warriach** **NO12** Assessment of ultrasonographic characteristics of ovarian antral follicles in progestogen- and eCG-treated ewes **Pawel Bartlewski** **NO13** FSH peak amplitude and ovarian follicular waves in ewes **Behzad Toosi** **NO14**	*Rupert Beckett Lecture Theatre, Michael Sadler Building*
17:00 – 18:00	*Registration*	*Foyer of Rupert Beckett Lecture Theatre, Michael Sadler Building*
19:00 – 20:00	SRF Welcome Reception	*Parkinson Court*
20:00 – 22:00	Postgradoc Quiz	*Senior Common Room*

PROGRAMME

TUESDAY 4TH JULY

09:00 – 11:00	**SRF Symposium 1: Endocrinology of Reproduction** **Chair: Bruce Campbell** GnRHs and GnRH receptors: Multiple roles in reproduction **Robert Millar** Ovulation: Novel genes and insights **JoAnne S Richards** Regulation of testicular function **Peter O'Shaughnessy**	*Rupert Beckett Lecture Theatre, Michael Sadler Building*
11:00 – 11:30	*Coffee Break*	*Parkinson Court*
11:30 – 13:00	Parallel Free Oral Communication Sessions 1 and 2 **Free Oral Communication Session 1: Reproductive Endocrinology** **Chairs: Robert Millar & Michelle Myers**	*Rupert Beckett Lecture Theatre, Michael Sadler Building*
11:30	Do women with PCOS have a more masculine brain? **Colin Duncan** **O01**	
11:45	Expression pattern of oviductal prostaglandin synthases changes during the estrous cycle **Cristoph Gabler** **O02**	
12:00	The effect of GnRH or hCG given on day 12 post-mating on ovarian function and embryo development of Beetal goat in Southern Punjab, Pakistan **Tasawar Khan** **O03**	
12:15	Treatment of early luteal phase ewes with anti progestagen **George Mann** **O04**	
12:30	The hormone relaxin and the primate cervix **Christina Simon** **O05**	

12:45	Leptin and Ovarian function: comparison of effects, mechanisms of action and application in different species **Alexandra Sirotkin** **O06**	
	Free Oral Communication Session 2: Uterus **Chairs: SK Dey & Samantha Llewellyn**	*Room LG19, Michael Sadler Building*
11:30	PLC-β1 deficient mice show impaired implantation **Panayiotis Filis** **O07**	
11:45	Arachidonic acid and uterine cyclooxygenase-2 **Anthony Flint** **O08**	
12:00	Expression of implantation markers in the uterus of the marmoset monkey **Kai Lieder** **O09**	
12:15	MMP-2 and -9, and TIMPs in equine endometritis **Charlotta Oddsdottir** **O10**	
12:30	Prostaglandin E_2 and $F_{2\alpha}$ regulation of cyclooxygenase-2 in endometrial adenocarcinoma cells via the F-series prostanoid (FP) receptor **Kurt Sales** **O11**	
12:45	Modulation of the Uterine Milk Protein in the Bovine Endometrium by Estradiol-17β **Susanne Ulbrich** **O12**	
13:00 – 13:30	*SRF AGM*	*Rupert Beckett Lecture Theatre, Michael Sadler Building*
13:30 – 14:30	*Lunch*	*Parkinson Court*

PROGRAMME

TUESDAY 4TH JULY (CONTINUED)

14:30 – 16:00	**SRF Student Prize Session** **Chair: Bob Robinson**	*Rupert Beckett Lecture Theatre, Michael Sadler Building*
14:30	The ubiquitin-proteasome pathway in murine oocytes **Chris Edgecumbe** **SP1**	
14:45	Does HCG inhibit Luteal MMP – 2 by increasing follistatin? **Michelle Myers** **SP2**	
15:00	A microdrop method for derivation of human embryonic stem cells **Behrouz Aflatoonian** **SP3**	
15:15	TFAM and POLG drive premature mtDNA replication in SCNT embryos **Emma Joanne Bowles** **SP4**	
15:30	Prokineticin 1: is it a regulator of endometrial function? **Jemma Evans** **SP5**	
15:45	Amino acid profiling of bovine oocytes **Karen Hemmings** **SP6**	
16:00 – 16:30	*Tea Break*	*Parkinson Court*
16:30 – 18:00	Parallel Free Oral Communication Sessions 3 and 4 **Free Oral Communication Session 3 – Male Reproduction and its effects on fertility** **Chairs: Eduardo Roladan & Kurt Sales**	*Rupert Beckett Lecture Theatre, Michael Sadler Building*
16:30	Intraspecific variation in sperm tail length in rodents **William Breed** **O13**	
16:45	Use of sex-sorted ram sperm in an embryo transfer program **Gareth Evans** **O14**	
17:00	Rapid determination of frequency of diploidy in human sperm **Tamas Kovacs** **O15**	

17:15	Leptin in Embryos sired by Males without accessory sex glands **Subin Liao** **O16**	
17:30	Histone and protamine compartments of human spermatozoa **David Miller** **O17**	
17:45	Seminal plasma regulation of cervical cytokine synthesis **David Sharkey** **O18**	
	Free Oral Communication Session 4 – Ovary **Chairs: JoAnne Richards & Rachel Webb**	*Room LG19, Michael Sadler Building*
16:30	cDNA cloning and expression of the human *NOBOX* gene **John Huntriss** **O19**	
16:45	CSF candidate Erp1/Emi2 during mouse oocyte maturation **Suzanne Madgwick** **O20**	
17:00	Whole ovary cryopreservation; the effects of Sphingosine-1-Phosphate inclusion **Vicki Onions** **O21**	
17:15	Local regulation of ECM remodelling in the rat ovary **Kirsty Shearer** **O22**	
17:30	Delayed TFAM and PolG activity reduces oocyte competence **Emma Spikings** **O23**	
17:45	BMP-6 mediated regulation of BMPR IB expression **Peter Marsters** **O24**	
19:30 – 00:00	**Conference Dinner** *Marshall Medallist 2006 to be awarded*	*Refectory*

PROGRAMME

WEDNESDAY 5TH JULY

09:00 – 10:00	**SRF Distinguished Scientist Lecture** **Chair: Helen Picton** Estrogen action in the ovary **Jock Findlay**	*Rupert Beckett Lecture Theatre, Michael Sadler Building*
10:00 – 11:30	**Attended Posters** *(including coffee)*	*Parkinson Court*
11:30 – 13:00	**SSR/SRF New Investigator Scientist Lectures** **Chair: Henry Jabbour** The Role of Gata Transcription Factors in Mammalian Reproductive Function **Robert Viger** Physiological control of angiogenesis in the bovine corpus luteum **Bob Robinson**	*Rupert Beckett Lecture Theatre, Michael Sadler Building*
13:00 – 14:00	*Lunch*	*Parkinson Court*
14:00 – 16:00	**SRF Symposium 2: Molecules of Reproduction** **Chair: Anthony Flint** Molecular cues of implantation **SK Dey** Reproductive roles of prolactin **Nadine Binart** Receptor cross talk and its implication in reproduction: the case of the GnRH receptor **Zvi Naor**	*Rupert Beckett Lecture Theatre, Michael Sadler Building*
16:00 – 16:30	Concluding remarks	*Rupert Beckett Lecture Theatre, Michael Sadler Building*

TIME	MONDAY 3RD JULY	TUESDAY 4TH JULY		WEDNESDAY 5TH JULY
09:00		**SRF Symposium 1** **Endocrinology of Repoduction** *Rupert Beckett LectureTheatre, Michael Sadler Building*		**SRF Distinguished Scientist Lecture** *Rupert Beckett Lecture Theatre, Michael Sadler Building*
09:15	**Registration** *Foyer of Rupert Beckett Lecture Theatre, Michael Sadler Building*			
09:30				
09:45				
10:00				
10:15				
10:30	**NOW Session 1** *Rupert Beckett LectureTheatre, Michael Sadler Building*			**Attended Posters (incl. coffee)** *Parkinson Court*
10:45				
11:00		**Coffee Break** *Parkinson Court*		
11:15				
11:30	**Coffee Break** *Parkinson Court*	**Free Oral Comm.** **Session 1 Reproductive** **Endocrinology** *Rupert Beckett LectureTheatre*	**Free Oral comm.** **Session 2 Uterus** **Room LG19** *Michael Sadler Building*	**SRF / SSR New Investigator Scientist Lectures** *Rupert Beckett Lecture Theatre, Michael Sadler Building*
11:45				
12:00				
12:15	**NOW Session 2** *Rupert Beckett Lecture Theatre, Michael Sadler Building*			
12:30				
12:45				
13:00	**Lunch** *Parkinson Court*	**SRF AGM** *Rupert Beckett LectureTheatre*	**Lunch** *Parkinson Court*	**Lunch** *Parkinson Court*
13:15				
13:30		**Lunch** *Parkinson Court*		
13:45				
14:00				
14:15	**NOW Session 3** *Rupert Beckett Lecture Theatre, Michael Sadler Building*			**SRF Symposium2 Molecules of Reproduction** *Rupert Beckett Lecture Theatre, Michael Sadler Building*
14:30				
14:45				
15:00				
15:15	**Tea Break** *Parkinson Court*	**SRF Student Prize Session** *Rupert Beckett Lecture Theatre, Michael Sadler Building*		
15:30				
15:45				
16:00		**Tea Break** *Parkinson Court*		**Close** *Rupert Beckett Lecture Theatre, Michael Sadler Building*
16:15	**NOW Session 4** *Rupert Beckett LectureTheatre, Michael Sadler Building*	**Free Oral comm.** **Session 3** **Male Reproduction** *Rupert Beckett LectureTheatre*	**Free Oral Comm.** **Session 4** **Ovary** *Room LG19, Michael Sadler Building*	
16:30				
16:45				
17:00	**Registration** *Foyer of Rupert Beckett LectureTheatre, Michael Sadler Building*			
17:30				
18:00				
18:30				
19:00	**SRF Welcome Reception** *Parkinson Court*			
19:30				
20:00	**Postgradoc Quiz** *Senior Common Room*	**Conference Dinner** *Refectory*		
20:30				
21:00				
21:30				
22:00				
22:30				
23:00				
23:30				
00:00				

PRIZE WINNERS' BIOGRAPHIES

MARSHALL MEDALLIST

Professor Anthony Flint

Tony Flint graduated from St Andrew's (Queen's College, Dundee) in Biochemistry in 1966 and from Bristol with a PhD in 1969. The subject of his PhD, which was taken in the Biochemistry Department, was carbohydrate metabolism in the ovary, and after graduating Tony took a postdoctoral fellowship at the University of Western Ontario, with Dave Armstrong. He returned to the UK in 1972, to a research position with Alec Turnbull, firstly at Cardiff, moving in 1973 with Alec to Oxford. At the John Radcliffe Hospital in Oxford, together with Anne Anderson, Tony described the changes in the ruminant placenta leading to parturition. Moving to the Babraham Institute in 1978 he subsequently showed how oxytocin secretion by the corpus luteum was involved in luteolysis, and how oxytocin receptor down-regulation by trophoblast interferon was responsible for the maternal recognition of pregnancy. In 1987 Tony was appointed to the Directorship of the Institute of Zoology in London, and in 1993 to the Chair of Animal Physiology at Nottingham. Tony's main interests have been in comparative physiology and the application of molecular biology to reproductive physiology. He has been a member of the SRF since 1973 (Secretary 1985 – 1989).

SRF DISTINGUISHED SCIENTIST

Professor Jock Findlay

Professor Jock Findlay is Deputy Director and Head of the Female Reproductive Endocrinology Group of Prince Henry's Institute of Medical Research, Melbourne, Australia and a Senior Principal Research Fellow of the National Health and Medical Research Council of Australia (NHMRC). He is Chairperson of the Infertility Treatment Authority of Victoria and the NHMRC Embryo Research Licensing Committee. Professor Findlay was Chair of the Scientific and Technical Advisory Group, Department of Reproductive Health & Research, World Health Organisation, Geneva, Switzerland, from 1998 – 2003.

His awards include the 1992 James Goding Memorial Lecture of the Australian Society for Reproductive Biology, the 1999 Society for Endocrinology (UK) Asia and Oceania Medal and the 2006 Dale Medal. He was made a Member of the Order of Australia (AM) in 2001 for services to medical research, particularly reproductive biology, and as a medical administrator.

Professor Findlay's research led to a paradigm shift in understanding the importance of local as well as peripheral regulation of endocrine tissues.

His laboratory has been funded by grants from the National Institutes of Health (USA), the Rockefeller Foundation (USA), the Welcome Trust (UK), and the NHMRC of Australia.

SSR/SRF NEW INVESTIGATOR SCIENTISTS

Dr Robert S Viger

Dr. Viger completed his Ph.D. in 1995 under the direction of Dr. Bernard Robaire at McGill University where he studied androgen action in the epididymis. He then joined the laboratory of Dr. Mona Nemer at the Institut de recherches cliniques de Montreal (IRCM) to obtain his postdoctoral training in molecular biology. While at the IRCM, Dr. Viger initiated studies on the role of GATA transcription factors in reproductive function, a topic which remains a major focus of his current research activities. Shortly thereafter, Dr. Viger moved to Quebec City and obtained a faculty appointment at Laval University. He is currently Associate Professor in the Department of Obstetrics and Gynecology and member of the Centre de Recherche en Biologie de la Reproduction (CRBR). During his still young research career, Dr. Viger has received numerous awards including the Governor General of Canada Gold Medal Award for his thesis work and outstanding trainee research awards from the Society for the Study of Reproduction (SSR) and American Society of Andrology. Dr. Viger is also the titleholder of a highly prestigious Canada Research Chair in Reproduction and Sex Development and was named recipient of the SSR New Investigator Award in 2005.

Dr Robert Robinson

Bob Robinson graduated from University of Bath in Biochemistry in 1996 which included a six-month placement at the University of Texas Medical Branch, Galveston, USA. He then completed his PhD in 2000 under the supervision of Professor Claire Wathes at the Royal Veterinary College, London where he studied the mechanisms involved in the establishment of early pregnancy in the dairy cow. During this time, he was awarded with the SRF student prize in 1997. From there, Bob worked for two years as a post-doctoral scientist at Pfizer in Sandwich, Kent in the Veterinary Medicine Discovery Biology department. For the last 4 years, he has been a post-doctoral researcher at the University of Nottingham working alongside Professor Morag Hunter, Dr George Mann and Professor Bob Webb. During this time, he has worked on two projects (1) Regulation of luteinisation and progesterone secretion in the bovine corpus luteum and (2) Identification of novel oocyte secreted proteins. Recently, he has obtained a Lectureship at the new University of Nottingham Veterinary School. Bob has been a member of SRF since 1996.

ABSTRACTS

NATIONAL OVARIAN WORKSHOP – SESSION ONE

Date: Monday 3 July 2006
Time: 10:30 – 11:30

NO1 Comparison of the bioactivities and follistatin-binding properties of activin-A, activin-AB and activin-B

Claire Glister, Phil G Knight

School Of Biological Sciences, AMS Building, University Of Reading, Whiteknights PO Box 228, Reading, UK

Introduction: Activins are disulphide-linked glycoproteins belonging to the TGFβ-superfamily. Homo- or hetero-dimerisation of two subunits (βA/βB) gives rise to three isoforms, activin-A (βAβA), activin-AB (βAβB) and activin-B (βBβB). There have been few studies to compare the biological actions of these three isoforms, and so we used (i) bovine theca cells to evaluate their relative bioactivity and (ii) BIAcore SPR to compare follistatin-binding properties.

Methods: Three independent batches of bovine theca cells, retrieved from 4-6mm follicles, were cultured under serum-free conditions (see Glister et al. 2005 Endocrinology 146:1883-1892) for 6 days with/without LH (50,150,4050 pg/ml) and with/without hrAct-A, hrAct-AB or hrAct-B (0,2,10,50 ng/ml). Media were changed every 48h and cell number determined at the end of culture. Conditioned media from the final period (96-144h) was assayed for androstenedione (A4) and progesterone (P4). BIAcore SPR was used to investigate the binding kinetics of each activin isoform for immobilized hrFS-288.

Results & Discussion: LH increased A4 output ($P<0.0001$) with a maximal (14-fold) response elicited by150 pg/ml. Maximal P4 output (32-fold) was elicited by much higher LH dose-levels (4050 pg/ml). LH dose-dependently reduced cell number (1.7-fold; $P<0.0001$). Activin-A suppressed basal and LH-stimulated A4 production (maximum 90% suppression; $P<0.001$). Activin-AB suppressed A4 secretion from cells under basal and low LH dose (50pg/ml) conditions (maximum 65% suppression; $P<0.01$) but had no effect on cells treated with higher LH doses. Activin-B did not affect basal or LH-stimulated A4 secretion. Basal and LH-induced P4 secretion were unaffected by any activin isoform. All three activins caused a modest increase in cell number ($P<0.005$). BIAcore analysis showed that activin-AB (KD 5×10^{-10}M) and activin-B (KD 6×10^{-10}M) bound to immobilised follistatin with ~50-fold higher affinity than activin-A (KD 10^{-9}M). This study reveals an intriguing dissociation between the bioactivity and follistatin-binding properties of activin isoforms that warrants further study.

Supported by BBSRC.

NO2 Purified follistatin isoforms from bovine follicular fluid (bFF) differ in their ability to bind and neutralise activin-A and associate with cell-surface proteoglycans

Claire Glister, Phil G Knight

School Of Biological Sciences, AMS Building, University Of Reading, Whiteknights PO Box 228, Reading, UK

Introduction: Follistatin (FS) is a high affinity binding protein for activin and several other TGFβ-superfamily members. Immuno-blotting of bFF reveals the presence of 5 different FS isoforms, generated by differential mRNA splicing and posttranslational modifications. It has been hypothesised that different FS isoforms show different binding kinetics for activin and for cell-surface proteoglycans giving rise to different biological properties and intrafollicular roles. To test this we purified FS isoforms from bFF and compared (i) their ability to neutralise activin-A bioactivity *in vitro* and (ii) their binding affinity for activin-A and heparin sulphate proteoglycans.

Methods: Native FS isoforms were isolated from bFF using a combination of immunoaffinity, reversed-phase and anion exchange chromatography. The bioactivity of each isoform was judged by its ability to reverse the suppressive effect of activin-A on LH-induced androgen secretion by cultured theca cells. BIAcore SPR was used to evaluate the binding kinetics of each isoform for immobilised activin-A and heparan sulphate proteoglycan.

Results & Discussion: Five different FS isoforms were isolated (>90% purity) from bFF (Mw 29, 31, 35, 37, 41kDa). Activin-A (10ng/ml) suppressed LH-induced androgen production by theca cells (>85% suppression). Each FS isoform was highly potent in reversing the suppressive effect of activin-A and, in support of our hypothesis, there were significant differences in potency. The 29kDa isoform had the greatest potency (almost identical to that of rhFS-288 also tested). The 35 and 41kDa isoforms had the lowest potency (~2-3 fold less). Using BIAcore SPR analysis, all FS isoforms bound effectively to activin-A, with the 35 and 41kDa isoforms binding most weakly. In contrast, only the 29kDa isoform (and rhFS-288) bound to immobilised heparan sulphate proteoglycan. These results support the hypothesis that naturally-occurring bovine FS isoforms differ in their ability to bind and neutralise activin-A and associate with cell-surface proteoglycans.

Supported by BBSRC.

NO3 Activin promotes *in vitro* preantral follicle growth

Marie McLaughlin

University Of Edinburgh, Institute Of Cell Biology, D502, Darwin Building, Kings Buildings, University Of Edinburgh, Mayfield Road, Edinburgh, UK

Introduction: Previous studies have demonstrated that activin influences murine and ovine follicle growth with both promotion and inhibition being observed *in vitro*. The aim of this study was to determine the effect of Activin A on somatic cell and oocyte development of bovine preantral follicles *in vitro*.

Methods: Preantral follicles (150 +/- 1μm) were isolated from bovine ovaries and cultured for 8 days in HEPES (20mM) buffered McCoy's 5a medium with bicarbonate, BSA (0.1%), glutamine (3mM), penicillin G (0.1mg/ml), streptomycin (0.1mg/ml), transferrin (2.5μg/ml), selenium (4ng/ml), insulin (10ng/ml) and ascorbic acid (50μg/ml), in the presence of human recombinant activin A at two concentrations (50 and 100 ng/ml) and the absence of activin (control). Half of the medium was replaced every fourth day at which time the follicle diameters were measured. On completion of the culture period follicles were fixed and processed for histology after which oocyte health and diameter were assessed.

Results & Discussion: Significant follicle growth was observed over 8 days in all groups ($p < 0.001$). Activin 100ng/ml, significantly increased follicle growth over control and activin 50ng/ml treatment groups by day 4 ($p < 0.05$). By day 8 of the culture period activin 100ng/ml significantly increased follicle growth over activin 50ng/ml treatment group ($p < 0.05$). Oocyte diameters significantly increased over time ($p < 0.05$) in all groups and by day 8 of the culture period follicles cultured in medium supplemented with activin 100ng/ml had a higher proportion of healthy follicles than control or activin 50ng/ml treated groups. Supplementation of the culture media did not significantly affect antral formation, which was observed to be highest in activin 100ng/ml treated group by day 8 of the culture period.

In summary activin promoted preantral bovine follicle growth *in vitro* however no concomitant follicle differentiation occurred over an 8-day culture period.

NO4 Bone morphogenetic protein-6 (BMP-6) greatly enhances gonadotrophin-dependent expression of mRNAs encoding the inhibin/activin subunits and gonadotrophin receptors in granulosa cells of preovulatory (F1-F3/4) chicken follicles

Sara L Al-Musawi, Richard T Gladwell, Philip G, Knight

School of biological Sciencs, University of Reading, Whiteknights, Reading RG6 6AJ, UK

Introduction: BMPs comprise a large subgroup of extracellular ligands belonging to the TGFβ, superfamily. Given the paucity of information regarding underlying mechanisms by which BMPs contribute to the regulation of ovarian function in birds, our aim was to investigate the *in vitro* effects of BMP-6 on granulosa cells from the domestic chicken.

Methods: Preovulatory follicles (F1, F2, F3/4) were dissected from hen ovaries (4/group for 3 replicate cultures) approximately 10 hours after predicted ovulation. Granulosa cells were isolated and cultured for 3 days. The effects of oLH and oFSH (10 and 100ng ml^{-1}) were tested in the absence/presence of rhBMP-6 (2, 10, 50ng ml^{-1}) under serum-free conditions. Conditioned media were analyzed for inhibin-A and progesterone by validated ELISA. Upon termination, real-time Q-PCR (TaqMan) was used to quantify mRNA expression for gonadotrophin receptors (LHR, FSHR), inhibin/activin subunits (α, βA, βB) and GAPDH.

Results & Discussion: Q-PCR showed that BMP-6 alone increased FSHR expression in F1 (2-fold; $P<0.01$), F2 (2-fold; $P<0.05$) and F3/4 (5-fold; $P<0.01$) cells while only enhancing LHR in F1 (1.5-fold; $P<0.01$) cells. BMP-6 greatly enhanced FSH-induced expression of FSHR (up to 10-fold; $P<0.01$) and LHR (up to 6-fold; $P<0.001$) in all follicles. BMP-6 also enhanced LH-induced LHR expression (up to 5-fold; $P<0.0001$) in all follicles without affecting LH-induced FSHR expression. BMP-6 dramatically enhanced FSH-induced expression of inhibin α (up to 65-fold; $P<0.0001$), inhibin/activin, βA (up to 10-fold; $P<0.05$) and inhibin/ activin βB (up to 40-fold; $P<0.0001$) subunits in all follicles. BMP-6 also enhanced LH-induced inhibin α mRNA expression in granulosa cells of F1 (12-fold; $P<0.01$), F2 (8-fold; $P<0.0001$) and F3/4 (7-fold; $P<0.0001$) follicles. These results firmly support the hypothesis that intra-ovarian BMPs have a key role in modulating granulosa cell function in the chicken.

Supported by BBSRC Committee.

NATIONAL OVARIAN WORKSHOP – SESSION TWO

Date: Monday 3 July 2006
Time: 12:15 – 13:00

NO5 11βHSD activities in porcine oocytes and cumulus-oocyte complexes.

Rachel Webb, Anthony Michael

Centre for Developmental & Endocrine Signalling, Academic Section of Obstetrics & Gynaecology, Division of CDS, St.George's (University of London), Cranmer Terrace, London SW17 0RE, UK

Introduction: Maturation of porcine oocytes can be inhibited by glucocorticoids. Within target cells, the actions of physiological glucocorticoids are modulated by 11β-hydroxysteroid dehydrogenase (11βHSD) enzymes which catalyse the inter-conversion of cortisol with its inert metabolite, cortisone. 11βHSD1 acts predominantly as an 11-oxoreductase enzyme to increase the local cortisol concentration, whereas 11βHSD2 act exclusively as a high affinity dehydrogenase to inactivate cortisol. Although expression of 11βHSD1 mRNA and protein have been reported in oocytes and cumulus cells (human and rat), enzyme activities have not been reported for oocytes or associated cumulus cells. The aim of this study was to establish whether porcine oocytes and cumulus-oocyte complexes (COCs) exhibit detectable 11βHSD activities and to establish the net direction of cortisol-cortisone metabolism.

Methods: Initially, groups of immature, compact COCs or mature, expanded COCs (10 COCs per 100μl medium) were incubated for 24h in medium 199 containing 100nM ^{3}H-cortisol. Subsequently, single compact COCs or denuded oocytes were similarly incubated for 24h in medium 199 containing 100nM ^{3}H-cortisol or ^{3}H-cortisone. Steroids were then extracted, resolved by thin layer chromatography, and quantified using a radiochromatogramme scanner.

Results & Discussion: Both compact COCs and expanded COCs exhibited high levels of cortisol oxidation. Net oxidation of cortisol was doubled in expanded COCs relative to compact COCs (7.4±1.1 *vs* 3.8±0.9pmol oocyte^{-1} 24h^{-1}, respectively, P=0.07) suggesting a possible increase in glucocorticoid inactivation as the porcine COC matures. Moreover, cortisol-cortisone inter-conversion was measurable in individual compact COCs and denuded oocytes. In single COCs and denuded oocytes, the rate of cortisol inactivation was 4-fold higher than the rate of cortisol regeneration (5.2±0.8pmol oocyte^{-1} 24h^{-1} vs 1.3±0.6pmol oocyte^{-1} 24h^{-1}, respectively). Hence, 11βHSD enzymes act predominantly in the porcine oocyte and surrounding cumulus cells to inactivate glucocorticoids. This may be important in limiting adverse effects of glucocorticoids on the maturation of porcine oocytes.

NO6 Ablation of core 1 o-glycan synthesis in the primordial oocyte enhances female fertility

Suzannah Williams[1], Lijun Xia[2], Rodger McEver[2], Richard Cummings[3] Rodger Pamela Stanley[1]

[1]*Department of Cell Biology, Albert Einstein College of Medicine, New York, NY*

[2]*Cardiovascular Biology Research Program, Oklahoma Medical Research Foundation, Oklahoma City, OK*

[3]*Department of Biochemistry and Molecular Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK*

Introduction: Protein glycosylation is required for many developmental processes. For example, modification of glycoproteins with core 1 O-glycans is essential for embryonic development beyond mid-gestation. Generation of these O-glycans requires T-synthase, which extends the Tn-antigen by the addition of galactose, to generate the T-antigen (Galβ1-3GalNAcα1-Ser/Thr). Subsequent modification of the T-antigen can generate core 2 O-glycans. We determined whether core 1 and 2 O-glycans have a role in oocyte development, fertilization and pre-implantation development in the mouse.

Methods: To ablate the T-synthase gene in primordial oocytes, female mice carrying both a T-synthase gene with exons 1 and 2 flanked by LoxP sites and a Cre recombinase transgene controlled by the oocyte specific promoter ZP3, were generated. Oogenesis, ovulation and embryonic development were examined.

Results & Discussion: Deletion of the T-synthase gene in oocytes was confirmed by genotyping, lectin staining of ovarian sections and eggs, and analysis of O-glycosylated ZP1 and ZP3. T-synthase$^{-/-}$ oocytes ovulate and are fertilized. Interestingly, homozygous T-synthase$^{F/F}$:ZP3Cre females produced significantly more pups (10.4 ± 3.1) than heterozygous females (6.5 ± 2.8; p = 0.0002). In addition, the time to first litter was 21.7 ± 1.4 days in the mutant compared to 24.3 ± 2.8 in heterozygous controls (p = 0.063). Analysis of ovulated eggs revealed that the ZP of mutant eggs appeared to be slightly thinner with a less defined edge compared to control eggs. However, the two ZP glycoproteins with O-glycans, ZP1 and ZP3, were both present in equal amounts in our analysis of 20 oocyte cumulus complexes, based on western blot analysis. As expected both ZPs exhibited a lower molecular weight than wild type counterparts. The combined data show that Core 1 and 2 O-glycans are not required for oogenesis, ovulation or fertilization and fertility appears to be enhanced in oocytes lacking these glycans.

NO7 The pro-apoptotic protein PDCD4 is a potential target for Dazl in mouse oocytes

Yvonne Brown[1], Kevin Morgan[1], Judy McNeilly[1], Nicola Reynolds[2], Howard Cooke[2], Wail Ismail[1], Alan McNeilly[1]

[1]*MRC, Human Reproductive Sciences Unit, Edinburgh, Scotland, UK*

[2]*MRC, Human Genetics Unit, Edinburgh, Scotland, UK*

Introduction: The RNA-binding protein Dazl is oocyte specific and in Dazl KO (-/-) mice ovaries appear normal at ed15 but lose all oocytes by d7pn (1). We have shown that HT (+/-) mice produce larger litters associated with increased follicle sensitivity to FSH compared to WT (+/+) mice. Despite this essential role for Dazl in the translational regulation of gene expression in female gametogenesis the targets have not been fully elucidated. The current research aims to determine potential target systems using the Dazl binding motif, to understand the role of Dazl in oocyte maturation.

Methods: Human and mouse RNA databases (National Centre for Biotechnology Information, NCBI) were analysed using BLAST software with the hypothesis that Dazl protein binds to the sequence (G/CUn)n (2). Matching sequences were categorised and catalogued according to motif length and protein function and RT-PCR was performed on mouse oocytes.

Results & Discussion: A number of potential target mRNAs containing (G/CUn)n were identified by screening approximately 8000 potential hits, among which was, programmed cell death 4 (PDCD4; MA-3). Both human and mouse PDCD4 mRNA possess a potential Dazl binding motif in the 3' untranslated region. We have now confirmed by RT-PCR that PDCD4 (3) and Dazl are co-expressed in mouse oocytes at the same stages, and are currently exploring their interactions if any. PDCD4 is a pro-apoptotic factor involved in transformation suppression (4). It is unclear what mechanisms are involved but it may affect oocyte survival by inhibiting apoptosis involving transcription factor AP-1. Changes in PDCD4 expression in oocytes is being quantified by RT-PCR and IHC during follicle development and its potential involvement in the loss of oocytes in the Dazl -/- mice is being explored. Other putative Dazl target genes in oocytes are now being explored *in silico* and by using immuno-pulldown (CLIP) analysis. [1]McNeilly et al (2000) *Endocrinology* 141, 4284-94, [2]Reynolds et al (2005) *Hum Mol Genet* 14, 3899-909, [3]Jurisicova et al (1998) *Mol Reprod Dev* 51, 243-253, [4]Cmarik et al (1999) *Proc Natl Acad Sci U S A* 96, 14037-42.

NATIONAL OVARIAN WORKSHOP – SESSION THREE

Date: Monday 3 July 2006
Time: 14:15 -15:15

NO8 *In vitro* maturation of sheep cumulus oocyte complexes using a serum-free maturation medium

Matthew Cotterill, Sally Catt, Helen Picton

Reproduction And Early Development Research Group, University of Leeds, D Floor, Clarendon Wing, Leeds General Infirmary, Belmont Grove, Leeds, UK

Introduction: Development of a physiological system for the study of oocyte function together with reports of large offspring syndrome in bovine and ovine species, and related epigenetic abnormalities in serum-base *in vitro* embryo production systems have prompted the development of serum-free systems for the *in vitro* maturation (IVM) of oocytes. The aim of this study was to compare IVM, fertilization and development of germinal vesicle (GV) oocytes to the blastocyst stage using serum-based and serum-free systems.

Methods: GV cumulus oocyte complexes (COCs) were isolated from sheep ovaries and matured for 24h at 39°C before *in vitro* fertilization (IVF) and embryo culture to the blastocyst stage. COCs matured in serum-free IVM medium comprised of αMEM basis containing 0.1% BSA (w/v), transferrin (5μg/ml), Pyruvate (0.047mM), Selenite (5ng/ml), glutamax (2mM), insulin (10ng/ml), long R3 IGF1 (10ng/ml), ovineFSH (100ng/ml) and ovineLH (100ng/ml) were compared to COCs matured in serum-based medium containing Glutamax (2mM), ovineFSH (5μg/ml), ovineLH (5μg/ml) and 10% sheep serum (v/v). COCs were cultured in groups of 25 in 500μl of medium. After 24h, cumulus expansion and mucification was recorded, IVF was conducted and zygotes were cultured, 5 zygotes/10μl Sydney IVF cleavage and blastocyst medium for a further 5 days at 39°C in 6% CO_2, 5% O_2 and 89% N_2. Maturation, cleavage rate and blastocyst development was recorded.

Results & Discussion: In total, 285 GV oocytes were matured and inseminated, 146 in a serum-free media vs 139 in serum-based media (92% serum-free vs 85% serum-based MII progression). Embryo cleavage rates (70.5% serum-free vs 75.7% serum-based), and blastocyst formation (28.1% serum-free vs 22.8% serum-based) were not significantly different ($p>0.05$) between treatments. In conclusion, these data demonstrate a serum-free media which can support equivalent rates of nuclear and cytoplasmic maturation in ovine oocytes which is comparable to a conventional serum-based system.

NO9 Bovine egg culture and vitrification using V1/V2a media

VC Moreira[1,2], A Ainslie[1], GJ McCallum[1], MJA Mylne[3], MJ Wood[2], TG McEvoy[1]

[1]*SAC, UK*

[2]*University of Aberdeen, UK*

[3]*Britbreed Ltd, East Lothian, UK*

Introduction: This study compared blastocyst yields from zygotes incubated in Synthetic Oviduct Fluid medium supplemented with BSA (4 g/l; SBSA) or in defined sequential media (V1/V2a) and subsequently investigated survival following vitrification in sealed CryoTips in the presence of SOF and V2a, respectively.

Methods: Presumptive zygotes (6 replicates; Fertilization = Day 0) were allocated randomly for culture from Day 1 onwards in SBSA or in V1 (to Day 4) and then V2a (Day 4 onwards). V1 differed from V2a in that it contained polyvinyl alcohol (3 g/l), EDTA (0.05 mmol/l), citrate (0.5 mmol/l) and taurine (0.1 mmol/l) and did not contain hyaluronic acid (0.5 g/l) or essential amino acids (BME, 50x; 20 ml/l). Blastocyst yields were recorded (Days 7 and 8) and those produced in SBSA (n=34) and V1/V2a (n=34) were vitrified in biosecure vials (CryoTip; Irvine Scientific) using SBSA and V2a as the respective Holding Media (HM). Vitrification solutions within the CryoTips comprised HM + 15% DMSO + 15% Ethylene Glycol + 0.5% Sucrose. Following storage in liquid nitrogen, and subsequent warming and cryoprotectant removal in SBSA or V2, survival was verified by blastocoele re-expansion. Data were analysed using Generalised Linear Models.

Results & Discussion: Culture of embryos in V1/V2a did not compromise blastocyst yields (SBSA *vs.* V1/V2a) on Day 7 (11+/-2.1 *vs.* 12+/-3.2%, ns) or Days 7+8 (19.5+/-1.75 vs. 18.1+/-2.84, ns). The V1/V2a system emulated a conventional cryopreservation regimen in terms of Day 7 blastocyst survival post-vitrification (SBSA *vs.* V1/V2a = 61+/-15 *vs.* 66+/-8%; ns). Overall (Day 7+8) survival rates were 25+/-4 *vs.* 46+/-10% for SBSA and V1/V2a, respectively (ns). Blastocyst survival following cryopreservation in sealed CryoTips indicates that a vitrification protocol incorporating a defined V2a formulation was applicable to blastocysts produced in defined sequential media.

SAC funded by SEERAD; VCM supported by UK Meat and Livestock Commission and Britbreed Ltd.

NO10 Effects of low physiological temperatures on pig oocyte nuclear maturation and kinase activity

Joanne Coleman, Jinpei Ye, Morag Hunter, Jim Craigon, Martin Luck

The School Of Biosciences , University of Nottingham, Sutton Bonnington Campus, Loughborough, Leicestershire, LE12 5RD, UK

Introduction: Studies using infrared technology found that temperature gradients exist within the ovary, particularly during the late follicular phase of the oestrous cycle. The maturing Graafian follicle is cooler than surrounding ovarian and abdominal tissues, by up to 2 deg C. This variation in temperature may influence oocyte development, including nuclear and cytoplasmic maturation, and we have found that nuclear maturation in pig oocytes is slower at lower temperatures. The present experiments investigated the effects of reduced temperature on enzymes which regulate the cell cycle, specifically MAP kinase and MPF.

Methods: Oocytes from gilt ovaries were synchronised by incubation in medium containing cycloheximide (CHX) for 16h. After removal from CHX, one set of oocytes was cultured for a further 24h at 39, 37 or 35.5C. A second set was matured at 39C for 8h and then at 37C or 35.5C for the remaining 24h. At 8 time points over the 24h period, oocytes were removed (20-25 per time point) and assessed for stage of maturation (GV, MI, MII) and MAPK and MPF activities. Enzyme activities were measured using an in vitro double kinase assay, with MBP and histone H1 as substrates.

Results & Discussion: Both nuclear maturation rate and kinase activity were significantly altered by culture for 24h at different, constant temperatures. Lower temperature delayed the rate of nuclear maturation by up to 4 hours. MAPK and MPF activities peaked at later time points but at the same stages of maturation. If oocytes were allowed to mature (by 8h incubation at 39C) beyond GVBD before the temperature was reduced, no effect on maturation rate or kinase activity was observed. We conclude that lower physiological temperatures decrease oocyte maturation rate and kinase activity but that oocytes are only sensitive to temperature prior to GVBD.

Supported by BBSRC.

NO11 APC^{cdh1} activity maintains germinal vesicle stage arrest of mouse oocytes

Alex Reis, Heng-Yu Chang, Mark Levasseur, Keith Jones

Institute for Cell and Molecular Biosciences, University of Newcastle,Newcastle, NE2 4HH, UK

Introduction: Mammalian oocytes remain arrested at the germinal vesicle (GV) stage within antral follicles until stimulated to enter the first meiotic division by a mid cycle luteinsing surge. This arrest is maintained by high protein kinase A activity, inhibiting Maturation-Promoting Factor (cyclin B1/CDK1) by affecting the phosphorylation status of CDK1. Here we examined the role of cdh1, an essential activator of the Anaphase-Promoting Complex (APC), which targets cyclin B1 for degradation.

Methods: GV mouse oocytes were microinjected with cRNA to 3 APC^{cdh1} substrates: cyclin B1, cdc20 and securin tagged to GFP. Their degradation was visualised by epifluorescence. To knockdown cdh1 we used a morpholino antisense (^{MO}cdh1). An inverted morpholino (inv^{MO}cdh1) was used as a control. To rescue cdh1 after knockdown cRNA to cdh1 was microinjected. Milrinone was used maintain GV arrest.

Results & Discussion: Cyclin B1-GFP induced GVB in GV-arrested oocytes. However rates were low, probably because we could observe its degradation following expression. If we inhibited the 26S proteasome or removed the degradation signal from cyclin B1, it became more effective at inducing GVB. We could also observe the degradation of two other APC^{cdh1} substrates: cdc20 and securin. Degradation of all 3 substrates was blocked by addition of a morpholino designed to knockdown cdh1 and could be rescued by further microinjection of cdh1 cRNA. A control inv^{MO}cdh1 was without effect. All these data seemed to suggest that APC^{cdh1} is active in GV oocytes. Interestingly APC^{cdh1} appeared to have a physiological function in maintaining GV arrest: ^{MO}cdh1 increased the rate of GVB by itself (30% GVB rates at 24 h), an effect which was further enhanced when co-injected with cyclin B1 (50% GVB rates). Therefore here we have described an important role for APC^{cdh1} in GV arrest.

NATIONAL OVARIAN WORKSHOP – SESSION FOUR

Date: Monday 3 July 2006
Time: 16:15 – 17:00

NO12 Characteristics of follicular waves and associated ovarian events during the oestrous cycle in Nili-Ravi buffaloes undergoing spontaneous and $PGF_{2\alpha}$ induced luteolysis.

Hassan Mahmood Warriach, Nasim Ahmad

Department of Theriogenology, Faculty of Veterinary Sciences, University of Veterinary and Animal Sciences, Lahore, Pakistan

Introduction: The objective of this study was to characterize the follicular waves and associated ovarian events during spontaneous and $PGF_{2\alpha}$ induced oestrous cycles in Nili-Ravi buffaloes.

Methods: Two experiments were conducted. In Exp.1, (n=12 oestrous cycles) follicular and luteal development was monitored by ultrasonography and jugular blood samples were collected simultaneously on alternate days. Buffaloes were monitored for ovarian follicles and corpora lutea (CL) from d 0 (first oestrus) until next oestrus. In Exp. 2, follicular dynamics were compared in buffaloes undergoing spontaneous (n = 12 oestrous cycles) and $PGF_{2\alpha}$ induced (n = 6) regression of the CL. Buffaloes were treated with two injections of 20 mg of $PGF_{2\alpha}$ i.m; 12 h apart on day 9 in order to induce luteolysis. These animals were observed for oestrus, two times a day using teaser bull.

Results & Discussion: Of 12 oestrous cycles, 9 (75 %) had two waves of follicular activity and only 3 (25%) had three waves during the oestrous cycle. The mean (± S.E.M) length of the oestrous cycle was shorter ($P<0.05$) in buffaloes with two waves than those with three waves (21.2 ± 0.1 vs. 22.8 ± 0.1 days). Mean day of emergence of the ovulatory wave was earlier ($P<0.05$) in two-wave oestrous cycles than in those with three waves (9.1 ± 0.3 vs. 11.3 ± 0.6 days). Mean growth rate of the ovulatory follicle was less ($P<0.05$) in oestrous cycles with two (0.63 mm/day) compared to three waves (0.93 mm/day). Mean onset of luteal regression was earlier (16.2 ± 0.2 vs. 18.6 ± 0.6 days; $P<0.05$) and the diameter of the ovulatory follicle on the same day was smaller in buffaloes with two waves than those with three waves (9.1 ± 0.3 vs. 11.0 ± 0.5 days; $P<0.05$). Mean numbers of small, medium and large follicles among various days of oestrous cycle were not different between two and three waves of follicular development ($P>0.05$). Area of the corpus luteum (CLA) was greater ($P<0.05$) and concentrations of progesterone in serum were higher ($P<0.05$) in buffaloes with three waves during days 14 through 20 compared to two waves of follicular development during the oestrous cycles. In Exp. 2, the dynamics of ovulatory follicular growth during the 3 days before oestrus were similar ($P>0.05$) in buffaloes undergoing spontaneous and $PGF_{2\alpha}$-induced luteolysis. CLA and serum concentration of progesterone were greater ($P<0.05$) during the 3 days before oestrous in buffaloes undergoing induced luteolysis as compared to the spontaneous luteolysis. The number of small and large follicles increased ($P<0.05$) and the number of medium follicles decreased ($P<0.05$) in buffaloes undergoing induced luteolysis as compared to those with spontaneous luteolysis. These results show that (1) majority of buffaloes had two wave pattern of follicular growth and emergence of a third wave was associated with a longer luteal phase (2) follicular dynamics during the 3 days before oestrus were similar in buffalos undergoing spontaneous and $PGF_{2\alpha}$ induced luteolysis.

NO13 Assessment of ultrasonographic characteristics of ovarian antral follicles in progestogen- and eCG-treated ewes

Pawel Bartlewski[1], Xinyu Liu[1], Qiao Dai[1], Ezra Hart[1], David Barrett[2], Norman Rawlings[2], Roger Pierson[3]

[1]*Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, Guelph, ON N1G 2W1, Canada*

[2]*Department of Veterinary Biomedical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK S7N 5B4, Canada*

[3]*Department of Obstetrics, Gynecology and Reproductive Sciences, College of Medicine, University of Saskatchewan, Saskatoon, SK S7N 0W8, Canada*

Introduction: The aim of this study was to examine the ultrasonographic characteristics of large ovarian follicles in cyclic and anestrous ewes that had received medroxyprogesterone acetate (MAP)-releasing sponges for 12 d, with or without an injection of 500 IU of eCG at sponge removal. We hypothesized that quantitative echotextural attributes of the follicles in ewes treated only with MAP would differ from those in MAP/eCG-treated ewes, reflecting the increased antral follicular development and secretory activity under eCG influence.

Methods: Digital images of ovulatory follicles from cyclic ewes and eCG-treated anestrous ewes (n = 32 follicles), and of anovulatory follicles ≥5 mm in control anestrous ewes (n = 8 follicles), were subjected to computerized image analysis. Image analyses utilized spot and line techniques designed to compare the ultrasonographic attributes of the follicular antrum, follicular wall and perifollicular ovarian stroma.

Results & Discussion: The mean diameter of ovulatory follicles increased ($P < 0.001$) 24 h after eCG treatment in cyclic ewes. The mean pixel intensity of the follicular antrum ($P < 0.001$), as well as mean pixel intensity and heterogeneity of the follicular wall and perifollicular ovarian stroma ($P < 0.05$), were greater in eCG-treated animals compared with control cyclic ewes 24 h after treatment. The mean diameter of ovulatory follicles in the eCG-treated anestrous ewes was greater ($P < 0.05$) than that of anovulatory follicles in control ewes after sponge removal. Pixel heterogeneity of the follicular antrum and follicular wall was greater ($P < 0.05$) in eCG-treated compared with control anestrous ewes 24 h after treatment. Our results support the hypothesis that large antral follicles in eCG-treated ewes exhibit distinctive echotextural characteristics. Follicular image attributes in eCG-treated ewes in both seasons appear to be indicative of the changes in follicular morphology and/or secretory function following the administration of the exogenous gonadotropin, which has both FSH- and LH-like activities.

NO14 FSH peak amplitude and ovarian follicular waves in ewes

Behzad Toosi, Srinivas Seekallu, Dave Barrett, Edward Bagu, Norman Rawlings

Department of Veterinary Biomedical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada

Introduction: In the ewe, ovarian antral follicular growth occurs in a wave-like pattern. A follicular wave is defined as the emergence or growth of 1 to 3 follicles from a pool of small follicles (1-3 mm in diameter) to achieve diameters of ≥5mm. There are 3 to 4 follicular waves per estrous cycle and each wave is preceded by a transient peak in circulating FSH concentration. The characteristics of the FSH peaks required for follicular emergence and growth have not been investigated in ewes.

Methods: In the present study, the effects of increasing FSH peak amplitude (4 to 5 times normal peak amplitude) on ovarian follicular wave dynamics were examined in cyclic Western White Face ewes. Seven ewes were given ovine FSH (1μg/kg, sc.) at the expected time of the endogenous FSH peak preceding the second antral follicular wave of the cycle. Ewes in the control group (n=6) received vehicle. Transrectal ovarian ultrasonography was done twice daily and blood samples were taken every 6 hours.

Results & Discussion: Increasing the amplitude of the FSH peak preceding a follicular wave did not alter the number of small and large follicles present in the ovary during the wave or the length of growth, static or regression phases of the follicular wave ($P > 0.05$). There was no significant difference in the number of follicles emerging in the wave, the interwave interval, maximum follicular diameter or follicular lifespan amongst the treatment and control groups ($P > 0.05$). There was a tendency for increased serum concentrations of estradiol in treated animals over the 7 day period after treatment (P=0.061). We concluded that although peaks in serum concentrations of FSH appear to be essential for follicular wave emergence, a 4 fold elevation of FSH peak amplitude did not affect the dynamics of the ensuing follicular wave.

SYMPOSIA SPEAKERS

SYMPOSIUM 1 – ENDOCRINOLOGY OF REPRODUCTION

Date: Tuesday 2 July 2006
Time: 09:00 – 11:00

S1 GnRHs and GnRH receptors: Multiple roles in reproduction

Robert P. Millar

MRC Human Reproductive Sciences Unit, The Queen's Medical Research Institute, 47 Little France Crescent, Edinburgh, EH16 4TJ, UK

Geoffrey Harris' seminal neuroendocrine demonstration of a hypothalamic humoral factor regulating pituitary reproductive hormones culminated in the structural elucidation of GnRH by Andrew Schally's laboratory. This set the scene for a remarkable acceleration in understanding GnRH biology and its diversity of functions and the development of analogues for clinical applications. Our group demonstrated the existence of different GnRH molecular structures in vertebrates. Currently 24 structural variants have been described in molluscs, protochordates and vertebrates representing some 500 million years of evolution. Cognate GnRH receptors have also been identified from all of these groups and the earliest-evolved GnRH receptor identified in a pair of neurosecretory motor neurons of C.elegans which integrate feeding and reproductive behaviour. This ancient role appears to have been remarkably conserved through to mammals as recently exemplified in the musk shrew and marmoset. In many vertebrates both GnRH and GnRH receptors have undergone gene duplications to give rise to a preoptic/hypothalamic GnRH I, the midbrain GnRH II and the forebrain GnRH III, each with its cognate receptor. In salmon these are co-ordinately activated on migration for spawning to stimulate the pituitary/gonads, reproductive behaviour, and olfactory systems respectively. In addition to central expression GnRH and GnRH receptors are also expressed in peripheral reproductive tissues (gonads, breast, prostate, uterus) where they appear to have autocrine/paracrine roles. In mammals only GnRH I, GnRH II and two cognate receptors are present. In man and certain other mammals the type II GnRH receptor has been silenced. However, both ligands can bind the type I GnRH receptor but stabilise it differently to couple differentially to intracellular signaling pathways. This phenomenon of "ligand-induced-selective-signaling" (LISS) is finding application in developing novel GnRH analogue therapeutics selective for targeting specific signaling in an array of normal and dysfunctional cells (eg cancers) which express GnRH receptors in the nervous system and peripheral tissues.

S2 Ovulation: Novel genes and insights

JoAnne S. Richards

Baylor College of Medicine, Houston, Texas, USA

Ovulation is a complex process initiated by the preovulatory LH surge, characterized by cumulus oocyte complex (COC) expansion and completed by the release of a mature oocyte. Although a critical role of the cumulus cells and the matrix formation has been documented, little is known about changes in the cumulus cell genetic program during ovulation. Therefore, a gene profiling approach was taken using RNA isolated from COCs collected from preovulatory follicles of eCG-primed mice and at selected times following hCG treatment. Microarray analyses and results were confirmed by RT-PCR analyses, Western blotting and immunofluorescent studies. A remarkable number of genes was up-regulated in COCs including the EGF-like factors, *Areg, Ereg,* and *Btc*. A few genes selectively expressed in cumulus cells compared to granulosa cells were related to neuronal (*Mbp, Tnc,*) cell function. Unexpectedly, a large number of genes expressed in COCs were related to immune cell (*Alcam, Pdcd1, Cd34, Cd52* and *Cxcr4, Runx1*) function, including pathogen surveillance receptors. Members of the Wnt/Fzd family (*Sfrp4, Fdz1 and Fdz2*), especially *Sfrp2* were induced/expressed in COCs. Thus, cumulus cells not only have a distinct cell fate (release from the ovary with the oocyte) but also express a unique set of genes. Furthermore, immunofluorescent analyses documented that cumulus cells are highly mitotic for 4-8h after hCG and then cease dividing in association with reduced levels of *Ccnd2* mRNA. Other down-regulated genes included: *Cyp19a1, Fshr, Inhb,* and the oocyte factors *Zp1-3* and *Gja4*. In summary, the vast number of matrix, neuronal and especially immune cell-related genes identified by the gene profiling data of COCs constitutes strong and novel evidence that cumulus cells possess a repertoire of immune functions that could be far greater than simply mediating an inflammatory-like response. Supported in part by NIH-HD-16229; HD-07495 (SCCPRR).

S3 Regulation of testicular function

Peter J. O'Shaugnessy

Institute of Comparative Medicine, University of Glasgow Veterinary School, Bearsden Road, Glasgow G61 1QH, UK

The adult testis functions primarily to generate spermatozoa and to secrete androgens. Both functions are dependent upon the somatic cells and, in particular, the Sertoli cells within the seminiferous tubules and the Leydig cells lying in the interstitial tissue. Early studies using hypophysectomised animals showed that activity of these cells was dependent upon luteinising hormone (LH) and follicle-stimulating hormone (FSH). More recently, use of mouse models lacking specific hormones or hormone receptors has allowed us to map out, more clearly, the role of each hormone in maintenance of testicular function. These studies have shown that the adult Leydig cell is critically dependent upon LH for both proliferation and androgen synthesis and, more surprisingly, that androgens themselves are required for normal Leydig cell function. Sertoli cells express receptors for both FSH and androgen and the relative roles of these two hormones in the regulation of spermatogenesis has long been a stimulus for debate. In so far as the mouse is concerned this has now been answered through studies on animals with targeted knockout of either the FSH- or androgen-receptor. Mice lacking FSH receptors have reduced testis size and sperm numbers but they are fertile. In contrast, in mice lacking androgen receptors spermatogenesis is arrested during meiosis indicating that androgen action is required for progression through this stage. Despite the clear, critical role for androgens in maintaining spermatogenesis little has been known until recently about the molecular mechanisms underlying androgen action on the Sertoli cell. Through use of microarrays and animal models of androgen insensitivity we are now, however, starting to identify targets of androgen action in the testis.

In contrast to the adult, the role of pituitary hormones in regulating fetal testicular function is less certain. In the mouse, both LH and adrenocorticotrophic hormone can stimulate fetal Leydig cell activity although fetal androgen production is normal in the absence of either hormone. Ablation of the fetal pituitary leads to loss of testicular steroidogenesis and it is possible, therefore, that both LH and ACTH regulate fetal Leydig cell function in a redundant manner.

SYMPOSIUM TWO – MOLECULES OF REPRODUCTION

Date: Wednesday 5 July 2006
Time: 16:30 – 18:00

S4 Molecular cues of implantation

S. K. Dey

Department of Pediatrics and Cell & Developmental Biology and Pharmacology, Vanderbilt University Medical Center, Nashville, TN 37232-2678, USA

The blastocyst implantation into the maternal uterus is a critical step in mammalian reproduction and, like many developmental processes, it involves a complex succession of genetic and cellular interactions. These interactions must be executed within an optimal time frame, termed window of implantation. Implantation in the uterus is a gateway to further embryonic development. It requires preparation of the uterus to the receptive state and implantation-competency of the blastocyst. This process is initiated by the attachment of the blastocyst to the uterine luminal epithelium. Concurrently with the attachment reaction, an increased endometrial vascular permeability occurs at the site of implantation. This is followed by localized endometrial decidualization with luminal epithelial apoptosis and subsequent invasion of the trophoblast into the stroma. Recent evidence suggests that a transient delay in the attachment reaction produces an adverse ripple effect throughout pregnancy producing aberrant development of the feto-placental unit with poor pregnancy outcome. Thus, on-time implantation is critical to normal feto-placental development. This constitutes a new concept that embryo-uterine interactions during implantation direct the subsequent developmental programming. This finding is clinically relevant, since implantation in humans beyond the normal "window" of uterine receptivity is correlated with higher risk of early pregnancy loss. The initiation of implantation and subsequent progression result from coordinated integration of various signaling pathways between the embryo and the uterus. Among the numerous signaling pathways associated with these events, growth factors, cytokines, prostaglandins, homeotic proteins and morphogens play major roles. A better understanding of periimplantation biology could alleviate female infertility and help to develop novel contraceptives.

S5 Reproductive roles of prolactin

Nadine Binart

INSERM 809 Hormone Targets, Faculté de Médecine – 156 Rue de Vaugirard – 75015 Paris, France

Prolactin (PRL) exerts pleiotropic physiological effects in various cells and tissues, and is mainly considered as a regulator of reproduction and cell growth. Null mutation of the PRL receptor (R) gene leads to female sterility due to a complete failure of embryo implantation. Pre-implantatory egg development, implantation and decidualization in the mouse appear to be dependent on ovarian rather than uterine PRLR expression, since progesterone replacement permits the rescue of normal implantation and early pregnancy. Alternative splicing of the prolactin receptor (PRLR) generates four isoforms, three short (RS) and one long (RL) form in mice. The mechanism by which PRL signals through the RL has been extensively investigated. However, little is known about PRL signaling through the RS. To ascertain the role of the RS in the ovary, PRLR null mouse model expressing only RS construct was generated. A premature ovarian failure was found in these mice. This ovarian phenotype is similar to phenotypes found in both Foxo3a null mice and in women carrying mutations of galactose-1-phosphate uridyl transferase (GALT). Foxo proteins are forkhead transcription factors known to regulate genes involved in glucose metabolism while GALT is an enzyme that converts galactose-1-P to glucose. Deficiencies in GALT lead to galactosemia and premature ovarian failure. To investigate whether Foxo3a expression is regulated by PRL signaling through the RS, ovaries from PRLR null and RS transgenic mice were subjected to microarray analysis. To determine whether Foxo3a regulates GALT expression, transfection studies were performed, they showed a marked up-regulation of the GALT promoter by Foxo3a. Foxo3a-GALT interaction provides a possible mechanism for the severe follicular impairment in females expressing RS. A better knowledge of the cellular and biochemical components involved in folliculogenesis and apoptosis should elucidate the mechanisms of premature ovarian failure.

S6 Receptor cross talk and its implication in reproduction: the case of the GnRH receptor

Zvi Naor

Zvi Naor[1,2], Henry N Jabbour[2], Michal Naidich[1], Adam J Pawson[2], Kevin Morgan[2], Sharon Battersby[2], Michael R Millar[2], Pamela Brown[2], Robert P Millar[2].

[1]Department of Biochemistry, The George S. Wise Faculty of Life Sciences, Tel Aviv University, Ramat-Aviv, Israel, 69978

[2]MRC Human Reproduction Sciences Unit, Centre for Reproductive Biology, The Queen's Medical Research Institute, Medical Research Council, Edinburgh, United Kingdom, EH16 4TJ.

The asynchronous phased secretion of LH and FSH during the reproductive cycle is a central dogma of reproduction, the mechanism of which remains unresolved. GnRH regulates its own receptors to enhance gonadotrope responsiveness during the cycle in preparation for the LH surge and ovulation, but the mechanism of the ensuing LH refractoriness during continued FSH secretion is not clarified. We now demonstrate that GnRH stimulates arachidonic acid (AA) release from the gonadotrope LβT2 cells via the Ca^{2+}-independent cytosolic phospholipase A_2 ($iPLA_2$) and not via the more common $cPLA_2\alpha$. AA release was followed by a marked induction of COX-1 and COX-2 by GnRH, which was mediated by the PKC/c-Src/PI3K/MAPK pathway and $iPLA_2$ but not via $cPLA_2\alpha$, or transactivation of the EGF receptor. COX1/2 act on AA to produce prostaglandins (PGs) and GnRH stimulates PGE_2, PGI_2 and $PGF_{2\alpha}$ production. We considered that these PGs may act in an autocrine manner to regulate gonadotrope function and demonstrate that rat pituitary gonadotropes express the prostanoids receptors EP1, EP2, FP and IP, while EP3 and EP4 were localized to the prolactin and growth hormone producing cells, respectively. $PGF_{2\alpha}$ and PGI_2 but not PGE_2 inhibit GnRH receptor expression through FP and IP receptors. The inhibitory effect of $PGF_{2\alpha}$ and PGI_2 seems to be mediated by inhibition of GnRH-stimulated phosphoinositide turnover. $PGF_{2\alpha}$ but not PGE_2 or PGI_2 reduced GnRH-induction of LHβ, but like PGE_2 and PGI_2 had no effect on the induction of common α, or FSHβ. $PGF_{2\alpha}$, or the COX1/2 inhibitor, indomethacin, inhibited and enhanced GnRH-induced LH secretion, respectively from rat pituitaries, but both had no effect on FSH secretion. PGE_2 and PGI_2 had no effect on LH and FSH secretion induced by GnRH. Hence, a novel inside-out signaling pathway mediated by $PGF_{2\alpha}$-FP and PGI_2-IP, acting in an autocrine/paracrine loop, limits GnRH regulation of the GnRH receptor, while $PGF_{2\alpha}$ inhibits also GnRH stimulation of LH but not FSH release. This mechanism may provide a means for the cyclical responsiveness of pituitary gonadotropes and the asynchronous LH and FSH release during the female reproductive cycle.

SRF STUDENT PRIZE SESSION

Date: Tuesday 4 July 2006
Time: 14:30 – 16:00

SP1 The ubiquitin-proteasome pathway in murine oocytes

Chris Edgecumbe[1], Marie Pantaleon[2], Peter Kaye[2], Michael D'Occhio[1]

[1]School of Animal Studies, University of Queensland, St Lucia 4072, Australia

[2]School of Biomedical Sciences, University of Queensland, St Lucia 4072, Australia

Introduction: Mammalian oocyte maturation is governed by a complex and dynamic interaction between protein kinases, phosphatases and proteases. Maturation promoting factor (MPF) is a serine/threonine kinase heterodimer composed of a catalytic cdc2/cdk1 subunit and a regulatory cyclin B1 subunit. Cyclin B1 undergoes rapid turnover via degradation by the ubiquitin-proteasome pathway (UPP) followed by *de novo* synthesis. A high level of MPF causes metaphase arrest, then UPP degradation of cyclin B1 allows the oocyte to exit metaphase I (MI). This study aimed to explore the subcellular distribution of key components of this dynamic system.

Methods: Confocal immunofluorescence microscopy was used to localise the 20S proteasome 'core' as well as one of the UPP substrates, cyclin B1 in mouse oocytes undergoing maturation *in vitro*. We also explored the localisation of fat facets in mouse (FAM), a deubiquitinylating enzyme, known to play a role in pre-implantation development.

Results & Discussion: Prior to maturation we find 20S proteasomal and cyclin B1 immunoreactivity is localised within the germinal vesicle (GV) whilst FAM is primarily found in the cytoplasm. Upon GV break down (GVBD), cyclin B1 and FAM were localised to the developing meiotic spindle. Interestingly, whilst 20S proteasome immunoreactivity was markedly decreased during GVBD, it appeared to localise to the spindle apparatus during MI and MII along with cyclin B1 and FAM. During anaphase-telophase I (AI/TI), all three proteins (20S proteasome, cyclin B1 and FAM) were localised between the dividing homologue chromosomes. Our data confirms that the 26S proteasome is required to localise with cyclin B1 during oocyte maturation in mouse oocytes. Moreover the presence and localisation of FAM to the same site suggests that together with the MAP kinase/$P90^{rsk}$ pathway, FAM may play a role in regulating levels of cyclin B1 during the AI/TI transition.

SP2 Does HCG inhibit luteal MMP-2 by increasing follistatin?

Michelle Myers, Eva Gay, Naomi S. Bulteel, W. Colin Duncan

Centre for Reproductive Biology, The Queens Medical Research Institute, The University of Edinburgh, Edinburgh, UK

Introduction: The expression and activity of human luteal MMP-2 is maximal during the late-luteal phase and inhibited by hCG during maternal recognition of pregnancy. As stromal fibroblasts, that are the primary source of MMP-2, do not express LH/hCG receptors, hCG must act through paracrine signalling mechanisms. Recently we have shown that activin-A up-regulates luteal fibroblast-like cell MMP-2 activity and expression. We therefore hypothesised that activin-A was a physiologically important paracrine regulator of luteal MMP-2.

Methods: Carefully dated human corpora lutea (CL) were examined by immunohistochemistry and quantitative RT-PCR. Luteal steroidogenic and fibroblast-like cells were derived from follicular aspirates collected at oocyte retrieval during assisted conception. The effect of hCG on cultures of steroidogenic cells and co-cultures of steroidogenic and fibroblast-like cells was assessed by immunoassay and quantitative RT-PCR.

Results & Discussion: In vivo, activin-A was localised to the steroidogenic cells, but not the stromal fibroblasts, of human CL. However the stromal fibroblasts expressed activin receptors, smad 2/3 and phospho-smad 2/3. Smad 2/3 increased during the luteal phase to a maximum in the late-luteal phase ($P<0.05$). In vitro, luteal steroidogenic cells express inhibin-A, activin-A and follistatin. The addition of hCG did not change the inhibin:activin ratio but increased follistatin expression ($P<0.05$). Furthermore, although hCG had no effect on fibroblast-like cell MMP-2 expression, it reduced the expression of fibroblast-like cell MMP-2 in co-cultures with steroidogenic cells ($P<0.02$). The addition of follistatin to co-cultures reduced MMP-2 expression in the absence of hCG ($P<0.01$). These results provide evidence that activin-A may be an important paracrine regulator of MMP-2 activity in the CL. In the late-luteal phase activin action and MMP-2 activity is increased. One of the effects of hCG in luteal rescue may be to inhibit the activin stimulation of MMP-2 by up-regulating follistatin expression.

SP3 A microdrop method for derivation of human embryonic stem cells

Behrouz Aflatoonian, Ludmila Ruban, Nick Jenkins, Ramya Udayashankar, Katie Amps, Harry Moore

Centre for Stem Cell Biology, University of Sheffield, Western Bank, Sheffield, S10 2TN, UK

Introduction: The conventional method for the derivation of human embryonic stem cells (HESCs) involves inner cell mass (ICM) co-culture with a feeder layer of inactivated mouse or human embryonic fibroblasts in an IVF culture dish. Growth factors potentially involved in primary derivation of HESCs may be lost or diluted in such a system. Our aim was to establish a microdrop method which maintained feeder cells and efficiently generated HESCs.

Methods: Embryos were donated for stem cell research after fully-informed patient consent. A feeder cell layer was made by incubating inactivated mouse embryonic feeder cells (MEFS) in a 50 µl drop of medium (DMEM/10%foetal calf serum) under mineral oil (Ovioil; Vitrolife) in a small tissue culture dish. MEFs formed a confluent layer and medium was replaced with HES medium supplemented with 10% Plasmanate (Bayer) and incubated overnight. Cryopreserved embryos were thawed (Thawing Pack; MediCult) and cultured (GIII+HSA; Vitrolife) until the blastocyst stage and the zona pellucida removed with 2 mg/ml pronase (Calbiochem). A zona-free intact blastocyst was placed in the feeder microdrop and monitored for ES derivation with medium changed every 2-3 days. Proliferating ES cells were passaged into other feeder drops and standard feeder preparation by manual dissection until a stable cell line was established.

Results & Discussion: Five HESC lines (SHEF3- 7) were derived by microdrop method. From a total of 46 blastocysts(early to expanded) five HESC lines were generated. SHEF 3 – 6 were generated on MEFs from 25 blastocysts. SHEF7 was generated on human foetal gonadal embryonic fibroblast (HGEF) from a further 21 blastocysts. From our experience microdrop technique is more efficient than conventional method for derivation of HESCs and it is much easier to monitor early ES derivation. The microdrop method lends itself to GMP derivation of HESCs.

SP4 TFAM and POLG drive premature mtDNA replication in SCNT embryos

Emma Joanne Bowles[1], Ramiro Alberio[2], Joon-Hee Lee[2], Rhiannon Lloyd[1], Keith Campbell[2], Justin St. John[1]

[1]*The Mitochondrial and Reproductive Genetics Group, The University of Birmingham, Birmingham, B15 2TT, UK*

[2]*Animal Development and Biotechnology Group, The University Nottingham, Sutton Bonington, Loughborough, LE12 5RD, UK*

Introduction: Somatic cell nuclear transfer (SCNT) involves introducing a fully differentiated cell into a recipient oocyte. The somatic cell nucleus is programmed to drive mtDNA transcription and replication, making it incompatible with oocyte mtDNA as these processes do not usually occur until the blastocyst stage. We hypothesise that expression of mitochondrial transcription factor A (TFAM) and DNA polymerase gamma (POLG) in embryos produced from SCNT could be premature.

Methods: In vitro matured sheep oocytes were fertilised to generate IVF embryos, or enucleated and fused with a single serum-starved sheep primary foetal fibroblast to generate NT embryos. Embryos were collected at different stages for RNA analysis and immunocytochemistry. For immunocytochemistry, embryos were fixed, blocked and stained for mtDNA-encoded COXI, and the nuclear-encoded TFAM and POLG and analysed by confocal microscopy. For transcript analysis embryos were frozen at -80ºC and analysed by real-time RT-PCR using Sybr Green to quantify levels of POLGA (catalytic subunit), POLGB (regulatory subunit) and TFAM transcripts. Transcript levels in the nuclear donor cells were also determined.

Results & Discussion: ICC revealed high levels of COXI, TFAM and POLG in IVF embryos at the 2-cell, morula and blastocyst stages with greatly reduced levels in 4 to16-cell embryos. In contrast, NT embryos expressed these proteins at consistently higher levels throughout pre-implantation development. Real time RT-PCR analysis showed low levels of TFAM, POLGA and POLGB transcripts in early stage IVF embryos with a significant increase to the morula and blastocyst stages, mirroring observed patterns of protein expression. Donor cells expressed TFAM, POLGA and POLGB transcripts, indicating an ability to drive mtDNA replication, when used in NT. The onset of mtDNA replication is tightly regulated but abnormally high levels of TFAM and POLG may result in the premature onset of mtDNA replication in NT embryos, thus disrupting normal development.

SP5 Prokineticin 1: is it a regulator of endometrial function?

Jemma Evans[1], K Morgan[1], HOD Critchley[2], RP Millar[1], HN Jabbour[1]

[1]*MRC Human Reproductive Science Unit, The University of Edinburgh, Centre for Reproductive Biology, Queen's Medical Research Institute, 47 Little France Crescent, Edinburgh EH16 4TJ, United Kingdom.*

[2]*Department of Reproductive and Developmental Sciences, The University of Edinburgh, Centre for Reproductive Biology, Queen's Medical Research Institute, 47 Little France Crescent, Edinburgh EH16 4TJ, United Kingdom.*

Introduction: The prokineticins are a novel family of angiogenic factors comprising prokineticin (PK) 1 and PK2. Their receptors are two closely related G-protein coupled receptors (GPCR) termed PKR1 and PKR2. This study was designed to investigate the expression and signaling pathways of PK1 and PKR1 in the human endometrium.

Methods: Ethical Approval for the study was obtained from the Lothian Research Ethics Committee and informed consent was obtained from all subjects prior to tissue collection. Expression of PK1 and PKR1 was investigated, by quantitative PCR and immunohistochemistry, in human endometrium collected across the menstrual cycle. In order to investigate the signaling pathways activated by PK1-PKR1 interaction, the Ishikawa endometrial epithelial cell line was stably transfected with the PKR1 cDNA in the sense direction. Subsequently, this cell line was used for analysis of signaling pathways that are activated in response to treatment with 10 nM recombinant PK1 either alone or in the presence of various chemical inhibitors of signaling molecules. Analysis of pathways was assessed by Western blot analysis.

Results & Discussion: PK1 expression varied across the menstrual cycle with highest expression detected in the late secretory phase ($P<0.01$); no variation in expression of PKR1 was detectable. Expression of PK1 and PKR1 was localized to the endometrial glandular cells and various cellular components of the stromal compartment including endothelial and immune cells. Stimulation of Ishikawa cells stably expressing PKR1 with 10 nM PK1 resulted in a dose and time dependent production of inositol phosphate ($P<0.01$) and phosphorylation of Src and ERK1/2. Incubation of PKR1 Ishikawa cells in the presence of chemical inhibitors of signaling molecules confirmed a role for PLC, PKC, Src and the EGF receptor in PK1 mediated phosphorylation of ERK1/2. These findings suggest that PK1 may play a role in endometrial function during the peri-implantation window and that it mediates its effect on target cells via activation of diverse signaling pathways.

SP6 Amino acid profiling of bovine oocytes

Karen Hemmings[1], Henry J Leese[2], Adam H Balen[1], Helen Picton[1]

[1]*Reproduction & Early Development Research Group, University of Leeds, LS2 9NS, UK*

[2]*Department of Biology, University of York, YO10 5YW, UK*

Introduction: The relatively low success rates and high incidences of multiple births following IVF has prompted the development of non-invasive amino acid profiling of Day 2-3 human embryos as a means to predict embryo developmental competence. Since embryonic genome activation does not occur until the 4-8 cell stage in humans, the metabolic profile exhibited by cleavage staged embryos reflects the metabolism of the oocyte. Therefore using bovine oocytes as a model for humans, we determined whether differences could be detected in amino acid consumption/ production between developmentally competent oocytes and those destined to arrest.

Methods: Abattoir derived bovine oocyte cumulus complexes were isolated and matured in serum-free *in vitro* maturation (IVM) medium for 16 hours at 39°C in 5% CO_2 in air. Denuded oocytes were cultured individually in 1μl microdroplets of a modified IVM medium for 6 hours to assess amino acid turnover before individual IVF was conducted. The competence of each presumptive zygote to develop to the blastocyst stage was then tracked in group culture by immobilizing each zygote onto the base of the petri dish using CellTak™ tissue adhesive. Zygotes were cultured in Sydney IVF media for 8 days at 39°C in 6% CO_2, 5%O_2, and 89% N_2. Spent culture media was analyzed for the amino acid content using a Kontron 500 series HPLC system.

Results & Discussion: Results from 157 oocytes showed a slight overall net appearance of amino acids during the culture period. Threonine was produced in significantly greater quantities in bovine oocytes that developed to the blastocyst stage (0.524+/-0.08pmol/oocyte/hour) compared to those which arrested (0.327+/-0.03pmol/oocyte/hour) ($P<0.05$). There was also a trend for developmentally competent oocytes to produce more lysine (0.501+/-0.19pmol/oocyte/hour) than those that arrested (0.203+/-0.11pmol/oocyte/hour) ($P=0.077$). These data suggest a link between amino acid metabolism and oocyte quality.

SRF DISTINGUISHED SCIENTIST LECTURE

Date: Wednesday 5 July 2006
Time: 09:00 – 10:00

P1 Estrogen action in the ovary

Professor Jock Findlay

Prince Henry's Institute, P.O. Box 5152, Clayton, Victoria 3168, Australia

Estrogen has a direct influence on folliculogenesis. Definitive evidence for its obligatory role and elucidation of its mechanisms of action on follicular cells comes from (a) identification of a second estrogen receptor, ERβ, the major form of ER in the ovary, and (b) production of mice with deletions of the ERα and/or ERβ, and *cyp*19 (aromatase) genes. These studies demonstrated that estrogen is (a) essential for fertility but not for survival or formation of the female reproductive tract, although estrogen could be required to establish the complete follicular reserve, (b) obligatory for normal folliculogenesis beyond the early antral stage and for ovulation, and (c) required for the ovarian phenotype of the somatic cells, possibly by an interaction with the oocyte. We hypothesized that estrogen regulates the genes responsible for somatic cell differentiation in the ovary. The relative expression of the following genes were increased in the ovaries of *cyp*19 deficient (ArKO) mice: ERβ, Sox 9, MIS, LRH-1, DAX-1, 17α hydroxylase, 17βHSD-1 and -3. ERα and LH receptor genes were decreased in ArKO ovaries. Treatment of ArKO mice with estradiol-17β for 3 weeks, increased LH receptor, decreased Sox 9, SF-1, 17α hydroxylase, and 17βHSD-1 and -3, did not affect the relative expression of ERα, ERβ, MIS, LRH-1 or DAX-1. These levels of gene expression are consistent with increased serum testosterone and the presence of Leydig and Sertoli cells in ArKO ovaries, and show that some of these genes are directly or indirectly regulated by estrogen. In conclusion, these studies clearly demonstrate a role for estrogen in somatic cell differentiation in the ovarian follicle.

Supported by NH&MRC of Australia (RegKey #198705 and 241000).

SSR/SRF NEW INVESTIGATOR SCIENTIST LECTURES

Date: Wednesday 5 July 2006
Time: 11:30 – 13:00

P2 The Role of Gata Transcription Factors in Mammalian Reproductive Function

Robert S. Viger

Ontogeny-Reproduction Research Unit, CHUL Research Centre

Centre de recherche en biologie de la reproduction (CRBR)

Department of Obstetrics and Gynecology, Faculty of Medicine, Laval University, Quebec City, Canada.

GATA factors were originally identified as crucial regulators of heart and haematopoietic cell development. These factors, however, are not unique to these two systems but rather are expressed in a variety of tissues including the gonads. Recent studies from our laboratory and other groups have implicated GATA factors in multiple reproductive processes including gonadal development, male sex differentiation, and steroidogenesis. Our insights into these roles have come mainly from the identification of novel GATA-dependent gene promoters. This growing list includes genes that code for hormones (AMH, INHA), steroidogenic enzymes (STAR, HSD3B2, CYP19A1), and transcription factors (DMRT1, SRY). Thus, GATA factors appear to play a central role in mammalian reproductive function. Their regulation and mechanism of action, however, have yet to be fully understood. The specificity of GATA action is controlled in part, via interactions with other transcriptional partners. Indeed, our original studies showed that GATA4 regulates *AMH* transcription in cooperation with steroidogenic factor 1 (SF-1). GATA4 also regulates *SRY* transcription through a similar cooperation, not with SF-1, but with Wilms' tumour 1 (WT1). Another mechanism for regulating GATA function is through post-translational modification of GATA proteins. We previously identified GATA4 as an effector of hormone action in gonadal cells via cAMP/PKA signalling and phosphorylation of GATA4. We now have evidence that GATA4 is also phosphorylated by MAP kinase but on a different set of amino acids. Elucidation of the in vivo role played by GATA factors in the gonads has been hampered by the embryonic-lethal phenotype of GATA knockout mice. To overcome this problem, we have generated a novel transgenic mouse model to knockdown GATA function in a tissue-specific manner. A description of this knockdown model and our other recent data on the role and regulation of GATA factors in reproductive function will be presented.

P3 Physiological control of angiogenesis in the bovine corpus luteum

RS Robinson, AJ Hammond, LT Nicklin, GE Mann and MG Hunter

School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough Leics, LE12 5RD UK.

It is widely recognized that luteal inadequacy is a major cause of infertility in a number of species. During the early luteal phase, progesterone production requires rapid growth of the corpus luteum (CL), which is in turn dependent on angiogenesis. While both fibroblast growth factor-2 (FGF-2) and vascular endothelial growth factor (VEGF) promote angiogenesis in the corpus luteum (CL), their precise role remains to be elucidated. In our investigations, we have clearly demonstrated that FGF-2 plays the more dynamic role during the transition periods in the bovine ovary such as the follicular-luteal transition and CL regression. Namely, FGF-2 production was up-regulated by the LH surge and during luteolysis. Furthermore, LH stimulated FGF-2 production by dispersed bovine luteal cells. Conversely, VEGF plays a more constitutive function in regulating angiogenesis in the pre-ovulatory follicle and CL. Collectively; this would indicate that it is FGF-2 which initiates these angiogenic processes, while VEGF is important in their development. Recently, we have developed a novel, primary and physiologically relevant culture system that mimics luteal angiogenesis. In this system, clusters of endothelial cells started to form on day 2, alongside isolated endothelial cells. Four days later, plaques formed with evidence of thread-like structures and by day 9 these tubule-like structures lengthened, thickened and formed highly organised intricate networks resembling a capillary bed. The endothelial cell network formation is influenced by FGF-2 and VEGF. Namely, VEGF increased VWF area by 4 fold on day 9, while FGF-2 alone had no effect. However, both FGF-2 and VEGF are required for maximal angiogenic response (10 fold increase, $P<0.001$). In conclusion, endothelial cell network formation was influenced by FGF-2 and VEGF and this novel culture system will enable the mechanisms involved in regulating luteal angiogenesis to be elucidated.

Funded by BBSRC.

FREE ORAL COMMUNICATION SESSION ONE – REPRODUCTIVE ENDOCRINOLOGY

Date: Tuesday 4 July 2006
Time: 11:30 – 13:00

Time: 11:30

O01 Do women with PCOS have a more masculine brain?

Elizabeth M Strachan, Colin Duncan

Reproductive and Developmental Sciences, The University of Edinburgh, Edinburgh, UK.

Introduction: Polycystic ovarian syndrome (PCOS) is a common and complex endocrine disorder of women with unknown aetiology. Evidence for a fetal origin of this condition is increasing. Female monkeys exposed to increased androgen concentrations in utero demonstrate the physical, metabolic and hormonal characteristics of PCOS in later life. There are clear gender differences in brain functions that can be assessed by questionnaires in adults. Testosterone may be involved in determining the maleness of the brain during fetal development. We hypothesised that women with PCOS may have been exposed to more androgen in utero and therefore have a more 'male' brain than women without PCOS.

Methods: We administered a validated questionnaire (Barron-Cohen) to assess 'maleness' of the brain to 197 women and 70 men, attending the Edinburgh Fertility and Reproductive Endocrine Centre and 256 were returned (96%). The diagnosis of PCOS was made on the basis of the Rotterdam consensus statement. Eight questionnaires had to be excluded as incomplete and 4 because there was not enough information to make the diagnosis of PCOS. Completed questionnaires were analysed from 88 women with PCOS, 93 women without PCOS and 62 men.

Results & Discussion: Women without PCOS (22.4; CI 20.5-25.8) had a lower systemising quotient than men (31.7; CI 28.7-34.7) ($p<0.001$, Kruskal Wallis), as had women with PCOS (23.1; CI 20.3-24.4) ($p<0.001$, Kruskal Wallis). There was no difference between women with PCOS and women without PCOS ($p>0.05$, Kruskal Wallis). Women with the highest, most male-like, systemising quotient were no more-likely to have PCOS. By assessing the maleness of the brain it is not possible to confirm the fetal origins of PCOS. However it is possible that postnatal differences have a role determining the male/female differences detected by this method.

Time: 11:45

O02 Expression pattern of oviductal prostaglandin synthases changes during the estrous cycle

Christoph Gabler, Simone Odau, Christoph Holder, Ralf Einspanier

Institute of Veterinary-Biochemistry, Freie Universität Berlin, Germany

Introduction: Prostaglandins (PG) are found in the bovine oviduct and were supposed to be involved in early reproductive events like fertilization and embryonic development. The aim of this study was to investigate the mRNA expression of specific enzymes synthesizing PGE_2, $PGF_{2\alpha}$, PGD_2 and PGI_2 in different oviductal regions during the estrous cycle.

Methods: Bovine oviducts were classified into four phases (pre-ovulatory, post-ovulatory, early and late luteal phase). Oviduct mucosa cells were harvested by flushing ampulla and isthmus separately. Total RNA was extracted and subjected to real-time RT-PCR. Immunohistological localization was performed using a specific antibody directed against microsomal PGE_2 synthase 1 (mPGES-1).

Results & Discussion: Quantitative RT-PCR revealed a 4-fold increase of mPGES-1 mRNA from the early luteal to the post-ovulatory phase. mPGES-1 mRNA content was higher in the isthmus than in the ampulla. Immunohistochemistry revealed that mPGES-1 was localized in the muscle and epithelium layer. In contrast, cytosolic PGES and mPGES-2 mRNA expression appeared unregulated during the whole estrous cycle. 20-α hydroxysteroid dehydrogenase (HSD) synthesizing $PGF_{2\alpha}$ was higher expressed in the isthmus after ovulation followed by a continuous decrease until the pre-ovulatory phase. However, HSD mRNA transcripts were found unregulated on low levels in the ampulla. PGI_2 synthase (PGIS) and lipocalin-type PGD_2 synthase (PGDS) were significantly higher expressed before ovulation compared with the post-ovulatory phase. PGIS and PGDS mRNA were several fold more abundant in the ampulla than in the isthmus. These findings reveal that synthases for PGI_2 and PGD_2 were up-regulated before ovulation possibly influencing the cumulus-oocyte-complexes in the ampulla. In contrast, synthases for PGE_2 and $PGF_{2\alpha}$ were higher expressed after ovulation in the isthmus indicating their important role for the transport and survival of the developing embryo.

Supported by DFG (Ei 296/10-2).

Time: 12:00

O03 The effect of GnRH or hCG given on day 12 post-mating on ovarian function and embryo development of Beetal goat in Southern Punjab, Pakistan

Tasawar Khan, Maria Rafeeq, Zaib Nisa, Mushtaq Lashari

Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan, Pakistan.

Introduction: Beetal is one of the famous breed of goat in Pakistan. Little is known about reproductive physiology of this goat. It has been demonstrated that a single injection of GnRH analogue or hCG, on Day 12 post-insemination can reduce embryo mortality in cattle and sheep. The effects of gonadotrophin supplementation in goats are not clear. The present study was initiated to determine the effects of GnRH or hCG on ovarian function and embryo development to improve litter size.

Methods: Twenty one goats were put to bucks at synchronized oestrus by two injections of $PGF_{2\alpha}$ given at 11 days apart. These animals were divided into three groups (7/group) through random stratification by body weight. These were given either saline (Group 1) or GnRH (Group II) or hCG (Group III) on day 12 post-mating. The blood samples were collected from 1 h before and 0, 2, 4, 6, 8, 24, 48 and 72 h after treatment for progesterone and oestradiol assays and were slaughtered on day 25 of pregnancy. Reproductive tracts were recovered, corpora lutea isolated, counted and weighed. Embryos were also recovered, weighed and measured for crown-rump length, amniotic sac length and width and numbers of caruncles forming placentomes were counted. The results showed that both GnRH and hCG increased plasma progesterone and oestradiol concentrations ($P < 0.05$) but hCG was stronger luteotrophic than GnRH. Treatments with GnRH and hCG not only improved conceptus growth but also increased number of caruncles significantly ($P < 0.05$). hCG proved to be stronger embryotrophic as compared with GnRH.

Results & Discussion: The results of this study support the hypothesis that gonadotrophin supplementation on Day 12 of pregnancy may improve embryo survival by enhancing conceptus growth and placentation as it is assumed that conceptuses with a significantly greater mass would tend to be more viable than smaller conceptuses.

Time: 12:15

O04 Treatment of early luteal phase ewes with anti progestagen

George Mann, Dawn Scholey, Morag Hunter, Robert Robinson

Division Of Animal Physiology, School Of Biosciences, Sutton Bonington Campus, Loughborough, University of Nottingham, LE12 6SY, UK

Introduction: Progesterone plays an important role in the regulation of luteolysis in the ewe. In this study we have investigated the effects of blocking progesterone action with the antiprogestagen onapristone during the early luteal phase on subsequent development of the luteolytic mechanism.

Methods: Cyclic ewes (n=8 per group) were treated from day 3 – 5 with the anti progestagen onapristone (100mg i.m. twice daily; ZK98299, Schering AG) or control vehicle. Ewes were then slaughtered on day 14 (n=4 per group) or day 17 (n=4 per group) and corpora lutea and uterine endometrium collected. On days 14, 15 and 16 frequent samples were collected to monitor PGF2α release episodes.

Results & Discussion: During treatment with onapristone circulating progesterone and oestradiol concentrations were unaffected while LH was elevated (7.50+/-1.47 vs. 1.60+/-0.24ng/ml; P<0.001). At slaughter, mean number of corpora lutea in treated ewes (5.0+/-0.4) was higher (P<0.001)

than in control ewes (2.3+/-0.3), due to the formation of accessory corpora lutea in the treated ewes. In ewes slaughtered on day 14, both oxytocin and progesterone receptor mRNA were undetectable in both groups. However, oestradiol receptor mRNA was elevated in treated ewes (0.12+/-0.01 vs. 0.06+/-0.01; P<0.05). During the 8h sampling windows on day 14, 15 and 16, the mean number of $PGF_{2\alpha}$ release episodes was higher in the control ewes (1.2+/-0.3 vs. 0.3+/-0.2 per 8h). Control ewes underwent luteolysis on day 15.8+/-0.2 while in treated ewes luteolysis had not occurred by slaughter on day 17. At slaughter on day 17, oxytocin receptor mRNA in the treated ewes was lower than control ewes (0.08+/-0.03 vs. 0.48+/-0.06 OD units; P < 0.01). These results show that treatment with onapristone early in the luteal phase advanced the appearance of uterine oestradiol receptor mRNA but delays the appearance of oxytocin receptor mRNA and the development of the luteolytic mechanism.

Time: 12:30

O05 The hormone relaxin and the primate cervix

Christina Simon[1,2], Almuth Einspanier[1,2]

[1]*Institute of Physiological Chemistry, Veterinary Faculty, Leipzig, Germany*

[2]*German Primate Center, Göttingen; Germany*

Introduction: The cervix plays an important role to protect the fetus against the outside milieu during pregnancy, but also to allow marked changes of the cervical connective tissue during parturition. This extreme reorganisation is under the control of hormones, like estradiol (E2) and relaxin (RLX). Therefore, the purpose of the present study was to examine the influence of RLX alone as well as compared to and in combination with E2 on the cervical tissue of the common marmoset monkey.

Methods: Two sets of experiments were carried out: i) intracervical diameter was analysed before and after the application of recombinant human (rh) RLX; ii) cervical tissue was collected from 4 groups of ovariectomized marmosets which were systemically hormone-treated: C (control group), E2 (35 µg/d), RLX (2×100 µg/d) and combination of both hormones. The cervical wet weight was then measured, the tissue histological and immunohistochemical analysed.

Results & Discussion: The results showed an increase of cervical diameter after the intracervical rhRLX treatment (5.0±1.2 vs. 5.8±1.0 mm). Furthermore a significant increase of the wet weights (0.25±0.04 vs. 0.1±0.04 g control) under hormone application was detectable. The histological analysis demonstrated a loosening of the extracellular matrix (ECM) of all treated groups versus control (E2+RLX>E2>RLX>C). These effects were associated with an increased number of eosinophile granulocytes as well as MMP-1 and MMP-2-expression. While steroid receptor expression was unchanged, the RLX-receptor LGR7 showed maximal expression in the RLX- and especially the E2+RLX-group.

In general, RLX has an effect on the ECM of the cervix confirmed by local as well as systemic application. However, the combined application of both hormones (E2+RLX) has a much stronger effect on the remodelling of the ECM at the primate cervix. Thus, it can be concluded that these hormones can act synergistically for effective softening of the cervix to support uncomplicated delivery.

Time: 12:45

O06 Leptin and ovarian function: comparison of effects, mechanisms of action and application in different species

A.V. Sirotkin[1, 2], R. Grossmann[3], A. Benčo[2], S. Pavlová[2]

[1]*Research Institute of Animal Production, 94992 Nitra, Slovakia*

[2]*Constantin the Philosopher University, 949 76 Nitra, Slovakia*

[3]*Institute for Animal Science (FAL), 31535 Neustadt, Germany*

Introduction: This presentation will review original data concerning the role of leptin (L) in the control of porcine, rabbit, human and chicken ovarian function (cell proliferation, apoptosis, secretion, oogenesis, ovulation, production and viability of pups), its extra- and intracellular mechanisms of action, and some practical applications of L in the control of reproductive processes. Whole animals, ovarian follicles and their fragments, ovarian granulosa cells or oocyte-cumulus complexes were treated with L, antiserum against IGF-I, blockers of protein kinases (PKA, MAPK, CDK), and gene constructs for transcription factors (CREB, STAT-1, p53). Markers of proliferation, apoptosis, hormone secretion, stages of meiosis etc. were evaluated by RIA, Western blotting, immunocytochemistry, TUNEL, cytogenetic and morphometric methods.

Methods: It was shown that L promotes apoptosis in porcine, rabbit, human and chicken ovarian cells and porcine cumulus oophorus. Furthermore, L stimulates proliferation in porcine, rabbit, and human ovarian cells but inhibits it in chicken ovarian cells. Leptin reduced plasma levels of hormones (steroids, IGF-I) in rabbit and the secretion of hormones (steroids, IGF-I, vasotocin) by cultured porcine, rabbit, human and chicken ovarian cells, and suppressed or promoted the response of ovarian cells to other hormones (gonadotrophins, IGF-I, ghrelin) in rabbit. It inhibited nuclear maturation in porcine, but not bovine oocytes and prevented maturation-associated decreases in MAP kinase in both porcine and bovine oocytes. Immuno-blockade of IGF-I prevented some effects of leptin in human ovarian cells. Leptin effects on rabbit, human and chicken ovarian cells and on porcine and bovine oocytes were associated with changes in PKA, MAPK and CDK in such cells, whilst blockers of these kinases prevented or promoted leptin action. Leptin affected the expression of transcription factors CREB, STAT-1 and p53 in porcine, rabbit and chicken ovarian cells, whilst transfection of porcine and rabbit granulosa cells with gene constructs for these transcription factors prevented or reversed L action. In-vivo experiments demonstrated that L could be used to predict reproductive efficiency and meat production in pigs and rabbits, for direct in-vitro control of maturation of pig oocytes and for in-vivo stimulation of reproduction in the rabbit.

Results & Discussion: These results suggest that L (1) is a stimulator of apoptosis (in all species studied), (2) regulates ovarian cell proliferation (stimulator in mammals, inhibitor in chicken), (3) is a regulator of ovarian hormone release (all species), (4) can regulate nuclear maturation, the state of cumulus oophorus and maturation factor MAPK (porcine and bovine), (5) can act through local production of IGF-I (in women, pig, rabbit and chicken), intracellular protein kinases PKA, MAPK and CDC2 (pig, rabbit, chicken, human), as well as through intracellular transcription factors CREB-1, STAT-1 and p53 (pig, rabbit, chicken), and (6) can be used to predict and control reproductive efficiency and performance (pig, rabbit) .

FREE ORAL COMMUNICATION SESSION TWO – UTERUS

Date: Tuesday 4 July 2006
Time: 11:30 – 13:00

Time: 11:30

O07 PLC-β1 deficient mice show impaired implantation

Panayiotis Filis, Lannagan, Alison Murray, Peter Kind, Norah Spears

University of Edinburgh, SBMS, Hugh Robson Building, George Square, Edinburgh, UK

Introduction: Mice that have a homozygous null mutation of the PLC-β1 gene show severely impaired fertility. In our colony, we have no record of knockout females giving birth. PLC-β1 is a phosphodiesterase that mediates multiple and diverse cell responses through the regulation of intracellular Ca^{2+} and phospholipid levels, however little is known about its roles in reproduction. PLC-β1 knockout females can produce fertilisation-competent oocytes in response to superovulation treatment (Lannagan and Spears, unpublished result), but they are unable to support pregnancy after fertilisation. The post-fertilisation block in mutant females was investigated to determine the role for PLC-β1 in early embryo development and implantation events in uterus

Methods: Knockout (KO) and control (wild type or heterozygous) females were mated with wildtype males and uteri and fallopian tubes of the females were examined 4.5 days after fertilisation. E4.5 (embryonic day 4.5) embryos from knockout females were found to have abnormal appearance and exhibit an implantation block in the uterus. We also found a dramatic reduction of implantation sites in uteri from KO females. Those implantation sites present failed to show the marked upregulation of the enzyme cyclo-oxygenase 2 (COX-2) that was exhibited by control animals. COX-2 is the key enzyme for the production of prostaglandins, which in turn is required for implantation.

Results & Discussion: We conclude that the implantation block of PLC-β1 knockout females may be attributed, at least partially, to inadequate production of prostaglandins at the sites of embryo implantation. Finally, we hypothesise that the abnormal morphology of KO embryos at E4.5 may be the result of inadequate or even detrimental cues provided by the reproductive tract of knockout females.

Time: 11:45

O08 Arachidonic acid and uterine cyclooxygenase-2

ELR Sheldrick[1], K Derecka[1], E Marshall[1], E Chin[2], DC Wathes[2], DRE Abayasekara[2], APF Flint[1]

[1]*School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, Leicestershire, LE12 5RD, UK*

[2]*Reproduction and Development Group, Royal Veterinary College, London, UK*

Introduction: Arachidonic acid is a paracrine message released by the uterine endometrial epithelium to induce COX-2 in the stroma. The mechanism by which it has this effect is not established.

Methods: A bovine endometrial stromal cell line was used to study the mechanism by which arachidonic acid induces COX-2 expression, using Western blotting and a reporter plasmid driven by the bovine COX-2 promoter.

Results & Discussion: COX-2 protein levels were induced by peroxisome proliferator-activated receptor (PPAR) ligands, including polyunsaturated fatty acids, synthetic PPAR agonists and non-steroidal anti-inflammatory drugs (NSAIDs). COX-2 levels were increased by the phorbol ester 4β-PMA, and the effects of arachidonic acid, NSAIDs and 4β-PMA were blocked by the PKC inhibitors RO 318425 and calphostin C. Arachidonic acid also increased expression of a COX-2 promoter reporter plasmid lacking a PPAR response element, showing that it also acted through a PPAR independent mechanism. This response was mimicked by 4β-PMA and $PGF_{2\alpha}$ but not by dibutyryl cyclic AMP or PGE_2 Induction of COX-2 reporter function by arachidonic acid was not blocked by the protein kinase C inhibitor RO318425, occurred in synergy with the effect of 4β-PMA and $PGF_{2\alpha}$, and therefore appeared to be independent of PKC. Induction of COX-2 protein by 4β-PMA (but not arachidonic acid) was reduced by the NF-κB inhibitors MG132 and parthenolide. Furthermore deletion of the NF-κB sites in the COX-2 promoter plasmid prevented the stimulation of reporter expression by 4β-PMA but not by arachidonic acid. These data are consistent with induction of COX-2 via PPARs. They also demonstrate that arachidonic acid induces COX-2 through a PPAR- and PKC/NF-κB-independent mechanism as well as via PPARs.

Time: 12:00

O09 Expression of implantation markers in the uterus of the marmoset monkey

Kai Lieder, Martina Kemper, Almuth Einspanier

Institute of Physiological Chemistry, Faculty of Veterinary Medicine, University of Leipzig, Germany

Introduction: Several factors are necessary to prepare a receptive endometrium. One of them is Relaxin (RLX), an important factor for connective tissue reorganisation, decidualization and angiogenesis. In the present study we have investigated the expression of RLX receptor LGR7, estrogen receptor alpha (ER) and enzymes of the estrogen biosynthetic pathway, like 17beta-hydroxysteroid dehydrogenase (17HSD) type 7 and 2, at different times of the reproductive cycle and early pregnancy, using the marmoset monkey.

Methods: Uterine tissue, collected by hysterectomy from cyclic and pregnant marmoset monkeys from day 9 to 16 of secretory phase (SP), was used for RT-PCR and immunohistochemistry (IC).

Results & Discussion: The expression of LGR7 mRNA was higher at the time of implantation (day 11/12 of SP) in conceptive uteri compared to non-conceptive uteri. This LGR7 expression is closely associated with elevated levels of peripheral blood levels of RLX as well as CG. The protein expression of RLX and its receptor LGR7 was mainly present in the deeper parts of the stromal endometrium at day 9/10 of SP shortly before implantation, whereas during implantation, RLX and its receptor were suddenly expressed in the luminal stromal and glandular regions of the endometrium, with major expression at implantation sites. 17HSD7 and 17HSD2 mRNA and protein expression increased on day 11/12 of SP (= implantation) as well, whereas the steroid receptor gene expression remained unchanged.

In summary, the up-regulation of the RLX receptor LGR7 around implantation suggests a role in modulating endometrial phenotype. At the same time, expression of 17HSD7 and 17HSD2 are up-regulated, suggesting a pre-receptor modulating action by influencing the concentration of highly bioactive estradiol, whereas the steroid receptor expression was unchanged. These findings seem to support the hypothesis that RLX acts in concert with estrogen to enhance factors which are required for implantation. Supported by grant application DFG Ei333/11-1

Time: 12:15

O10 MMP-2 and -9, and TIMPs in equine endometritis

Charlotta Oddsdottir[1], Simon C Riley[2], Rosemary Leask[2], Elaine D Watson[1]

[1]*Division of Veterinary Clinical Studies, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush Veterinary Centre, Midlothian, EH25 9RG, UK*

[2]*Obstetrics and Gynaecology Section, Queen's Medical Research Institute, University of Edinburgh, UK*

Introduction: Tissue remodelling normally occurs in the endometrium without scarring due to processes involving the matrix metalloproteinases (MMPs) and their inhibitors (TIMPs). We hypothesise that endometrial fibrosis is a result of scar tissue formation following endometritis.

Methods: Low volume (60 ml) uterine flushes were collected from mares (n=5) in oestrus and dioestrus, and during bacterial and mating induced endometritis. Gelatin zymography and reverse zymography were performed to assess activities of MMP-2 and -9, and TIMPs, respectively.

Results & Discussion: Basal activities of MMP-2 and MMP-9 did not differ between oestrus and dioestrus. An increase ($p<0.05$) was detected in MMP-2 and MMP-9 activity during endometritis in oestrus and dioestrus. Basal TIMP-2 activity in controls was higher ($p<0.05$) in dioestrus than in oestrus and activity decreased ($p<0.05$) during endometritis in dioestrus. MMP-2 and -9 are likely to play a role in tissue remodelling during endometrial repair and endometritis and may be modulated by TIMP-2. The control of theses enzymes may be important in the pathophysiology of endometritis and endometrial fibrosis.

Time: 12:30

O11 Prostaglandin E_2 and $F_{2\alpha}$ regulation of cyclooxygenase-2 in endometrial adenocarcinoma cells via the F-series prostanoid (FP) receptor

Kurt Sales, Henry Jabbour

MRC Human Reproductive Sciences Unit, The Queens Medical Research Institute, 47 Little France Crescent, Old Dalkeith Road, Edinburgh, EH16 4TJ, UK

Introduction: Prostaglandin $F_{2\alpha}$ receptor (FP receptor) expression and signalling is upregulated in endometrial adenocarcinomas. The endogenous ligand for these receptors is considered to be $PGF_{2\alpha}$, however PGE_2 can bind to the FP receptor with an affinity that is only 30 fold less than $PGF_{2\alpha}$. This study was designed to investigate the activation of intracellular signal transduction pathways and target genes by PGE_2 and $PGF_{2\alpha}$ via the FP receptor.

Methods: The FP receptor was stably transfected into Ishikawa endometrial adenocarcinoma cells (FPS cells). Total inositol phosphatide generation in FPS cells was measured by formic acid chromatography. Intracellular signalling and target gene activation by PGE_2 and $PGF_{2\alpha}$ in FPS cells was measured by luciferase reporter assay.

Results & Discussion: Treatment of FPS cells with PGE_2 or $PGF_{2\alpha}$ dose dependently mobilised inositol 1,4,5 trisphosphate, promoted the expression of the cis-acting enhancer elements, activator protein-1 (AP1), cAMP response element (CRE), heat shock response element (HSE) and serum response element (SRE) and induced the expression of the COX-2 promoter. Co-incubation of FPS cells with the specific FP receptor antagonist AL8810 abolished the effects of $PGF_{2\alpha}$ on the activation of the cis-acting enhancer elements and COX-2 promoter and significantly reduced the effect of PGE_2 on the activation of the cis acting enhancer elements and COX-2 promoter. In combination, PGE_2 and $PGF_{2\alpha}$ synergistically elevated the expression of the cis-acting enhancer elements and COX-2 promoter via the FP receptor to a greater extent than each ligand on its own. These data indicate that PGE_2 and $PGF_{2\alpha}$ can converge on the FP receptor in endometrial adenocarcinoma cells to augment the activity of transcriptional activators and target genes involved in tumorigenesis.

Time: 12:45

O12 Modulation of the uterine milk protein in the bovine endometrium by estradiol-17β

Susanne E. Ulbrich[1], Karin Gross[2], Susanne Schmidt[3], Helmut Blum[3], Regine Rottmayer[2], Stefan Hiendleder[2], Eckard Wolf[2,3] Fred Sinowatz[4], Heinrich HD Meyer[1], Stefan Bauersachs[2,3]

[1]*Physiology Weihenstephan, Technical University Munich, Freising, Germany*

[2]*Institute of Molecular Animal Breeding, Gene Center of the Ludwig Maximilians University, Munich, Germany*

[3]*Laboratory for Functional Genome Analysis (LAFUGA), Gene Center of the Ludwig Maximilians University, Munich, Germany*

[4]*Institute of Veterinary Anatomy, Histology and Embryology, Ludwig-Maximilians-University Munich, Germany*

Introduction: Uterine secretion from the endometrial glandular epithelium is necessary for successful fertilization and early embryonic development. A specific immunomodulatory role has been subscribed to the uterine milk protein (UTMP), a member of the serine proteinase inhibitor superfamily. A detailed characterization of *UTMP* mRNA was undertaken by analyzing a number of time points during the estrous cycle and the pre-implantation period in different segments of the bovine endometrium. In addition, effects of steroid hormones were investigated using a bovine endometrial cell culture.

Methods: Simmental heifers were slaughtered at estrus and 3.5, 12, and 18 days after estrus in and additionally at day 15 and 18 of pregnancy, respectively (n=4 per group). The uterus was divided into corpus and caudal, middle and cranial parts of the ipsilateral uterine horn. Samples were collected from the intercaruncular endometrium. *UTMP* mRNA was quantified by real-time RT-PCR and immunoreactive protein was localized by immunohistochemistry. Additionally, an endometrial *in vitro* culture (n=4) was stimulated with physiological doses of progesterone or estradiol-17β.

Results & Discussion: Remarkable changes of *UTMP* mRNA occurred during the estrous cycle. The most pronounced expression was at day 18 and estrus in the glandular epithelium followed by a dramatic decrease at day 3.5. Pregnant animals revealed a lower expression of UTMP at day 18 as compared to the cyclic heifers, the latter already progressing towards estrus. The potent stimulatory influence of estradiol-17β on UTMP was additionally shown *in vitro*. The pronounced gradient from the cranial uterine horn to the corpus at estrus was modulated at day 3.5 and absent at days 12, 15 and 18 indicating functional implications regarding the passing gametes and the early embryo. By providing a well-prepared endometrial environment UTMP seems to play a decisive role in mechanisms of early embryo-maternal communication.

Supported by DFG FOR 478.

FREE ORAL COMMUNICATION SESSION THREE – MALE REPRODUCTION & ITS EFFECTS ON FERTILITY

Date: Tuesday 4 July 2006
Time: 16:30 – 18:00

Time: 16:30

O13 Intraspecific variation in sperm tail length in rodents

William Breed[1], Melissa Bauer[1], Rebecca Wade[1], Nattawut Thitipramote[2], Jintamas Suwajarat[2], Lisa Yelland[3]

[1]*Discipline of Anatomical Sciences, Faculty of Health Sciences, The University of Adelaide, Adelaide, South Australia*

[2]*Department of Biology, Faculty of Science, Prince of Songkla University, Songkla, 90110, Thailand*

[3]*Discipline of Public Health, Faculty of Health Sciences, The University of Adelaide, Adelaide, South Australia*

Introduction: Eutherian mammals exhibit marked interspecific differences in sperm head shape and tail length with a few species having sperm head pleiomorphism. Intraspecific variability in sperm tail length has, however, rarely been suggested. Here we ask the question: Do murine rodent species that have highly pleiomorphic sperm head shapes show intra-individual variability in sperm midpiece and total tail lengths as well? For this we selected the following three pairs of species from three separate lineages one of which has monomorphic, and the other highly divergent and pleiomorphic, sperm head shapes-*Bandicota bengalensis* and *B. indica* from southern Asia, *Aethomys chrysophilus* and *A. ineptus* from southern Africa, and *Pseudomys australis* and *Notomys* alexis from Australia.

Methods: Cauda epididymal sperm smears were prepared and sperm midpiece and total tail lengths determined from about 200 sperm from each species by light microscopy. A linear mixed effects model was used to compare intra-individual variability between the pairs of species investigated.

Results & Discussion: The findings show that in all three species with pleiomorphic sperm heads (*B. indica, A. ineptus* and *N. alexis*), statistically significant greater intra-individual variability of sperm midpiece and total tail lengths were evident ($p<0.0001$ in all cases). Regression analysis of testis mass against body mass indicated that these species also have relatively small testes mass (residuals for *B.indica* –1.01, for A. *ineptus* –0.75, and for *N. alexis* -2.42) probably reflecting low levels of intermale sperm competition perhaps due to a monogamous mating system. The findings thus suggest that sexual selection may affect both intraspecific variability of gamete shape and size as well as relative testes mass. Depressed levels of intermale sperm competition may thus result in both the evolution of divergent and pleiomorphic sperm head shapes as well as variable midpiece and total tail lengths in this group of mammals.

Time: 16:45

O14 Use of sex-sorted ram sperm in an embryo transfer program

Simon P de Graaf, Kiri H Beilby, Justine K O'Brien, WMC (Chis) Maxwell, Gareth Evans

Reprogen, Faculty of Veterinary Science, The University of Sydney, Sydney, Australia, NSW 2006

Introduction: Preselection of sex of offspring has been realised through the use of flow cytometrically sorted sperm within artificial insemination and *in vitro* fertilisation programs. Little is known about the feasibility of combining sex-sorting technology with multiple ovulation and embryo transfer (MOET). This study was conducted to determine the functional capacity of sex-sorted ram sperm in a MOET program.

Methods: Semen was collected from two White Dorper rams, and split into two parts for sex-sorting and freezing (SF) or standard freezing (unsorted Control). Intrauterine inseminations were conducted using commercial laparoscopic techniques on superovulated mature White Dorper ewes (N=70) in synchronised oestrus utilising either SF sperm (15×10^6 motile sperm/ewe; SF_{15}) or Control sperm (15×10^6 or 30×10^6 motile sperm/ewe; C_{15} or C_{30}, respectively). Resultant embryos and unfertilised ova were flushed at Day 6 and morulae and blastocysts were transferred immediately into synchronised recipients (N=160). Pregnancy was diagnosed at Day 60 by real-time cutaneous ultrasound.

Results & Discussion: The fertilisation rate of SF_{15} sperm (172/230; 74.8%) was significantly higher ($P < 0.05$) than that of C_{15} (97/151; 64.2%) or C_{30} (89/141; 63.1%) sperm. However, no significant difference ($P > 0.05$) existed between the percentages of embryos transferred per treatment group. The percentage of embryos transferred which resulted in a fetus at ultrasound did not differ significantly between SF_{15} (98/124; 79.0%), C_{15} (59/74; 79.7%) or C_{30} (59/70; 84.3%) treatment groups ($P > 0.05$). These are the first recorded pregnancies in sheep as a result of combining sex-sorting technology with multiple ovulation and embryo transfer. The similar levels of fertilisation and embryo survival recorded for sex-sorted and control groups suggest that the function of sex-sorted ram sperm is not impaired, and nor is the developmental capacity of the resultant embryos reduced.

Supported by XY Inc. & Allstock Technology.

Time: 17:00

O15 Rapid determination of frequency of diploidy in human sperm

Tamas Kovacs[1], Gyongyi Bekesi[1], Attila Jenei[2], Margit Balazs[3], Laszlo Matyus[2]

[1]*Dept. of Obstetrics and Gynaecology, Medical and Health Science Center, University of Debrecen, Hungary*

[2]*Department of Biophysics and Cell Biology, Research Center for Molecular Medicine, Medical and Health Science Center, University of Debrecen, Hungary*

[3]*Department of Preventive Medicine, School of Public Health, Medical and Health Science Center, University of Debrecen, Hungary*

Introduction: Commonly used parameters of sperm quality do not correlate with fertility in all instances. It has been shown that elevated frequency of chromosomal abnormalities in sperm is associated with decreased fertility. However, investigations are time-consuming and work-intensive. In our study we examined if flow cytometry is suitable for detection of abnormal chromosomal content of human spermatozoa.

Methods: Semen samples from 6 normozoospermic men were washed then treated with the propidium iodide based DNA Prep Reagent Kit (Beckman Coulter, Fullerton, CA), and decondensed using lithium-diiodosalicylate. Flow cytometry was performed on a Digital Vantage flow cytometer (Becton Dickinson, San José, CA, USA) and diploid cell population was quantified based on relative fluorescence intensity. Diploids were separated in sorter mode, and triple color fluorescent in situ hybridization was performed both on the sorted and the original semen as described by us elsewhere.

Results & Discussion: 1-2 x 10^6 spermatozoa were processed from each sample. Proportion of cells identified as diploids varied between 2.1 and 3.8%. FISH of the original semen revealed a frequency of 0.15-0.44% of diploid sperm, while in the sorted samples it ranged between 5 and 21%. There was a strong correlation between the proportion of sorted cells to the total cell number and the frequency of diploids in the original semen specimen ($p<0.001$, Spearman correlation).

Flow cytometry is suitable for rapid measurement of diploidy frequency in human sperm. Diploidy rate is closely related to the occurrence of meiotic errors during spermatogenesis, and so to fertility. By introducing this method in semen analysis more direct data can be obtained on the etiology of male infertility than by conventional semen analysis. Detection of disomies of the large chromosomes (group A, B) would be also realistic in the future.

This work was supported by the grant of Hungarian Ministry of Health (ETT 534/2003).

Time: 17:15

O16 Leptin in embryos sired by males without accessory sex glands

Subin Liao[1], P.H.Chow[2], P.L.Cheung[1], W.S. O[1]

[1]*Department of Anatomy, Faculty of Medicine, The University of Hong Kong, Hong Kong*

[2]*Department of Anatomy, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong*

Introduction: Our previous studies showed that embryos sired by golden hamsters with all accessory sex glands (ASG) removed exhibited anomalous development. As leptin, the *obese* gene product, plays an important role in embryonic development, we aim to find out the leptin expression in embryos sired by males with all accessory sex glands ablated.

Methods: Male hamsters with all ASG ablated (TX) and sham-operated controls (SH) were hand mated with cycling females on the day of oestrus. Embryos at 2-, 4-cell and blastocyst stages and conceptus at 7, 9 and 11 *dpc* (days post coitus) were collected. Leptin mRNA in pre- and post-implantation embryos was detected by RT-PCR. Leptin in pre-implantation embryos were stained with immunofluorescence method and the relative intensity measured from confocal images. Protein in postimplanation embryos were measured by ELISA.

Results & Discussion: Differing from the mouse, leptin but not its mRNA was detected in the hamster pre-implantation embryos. The relative immunofluorescence intensity of leptin in TX embryos was significantly lower than SH (TX 0.758+/- 0.048 vs SH 0.870+/-0.052, $P<0.05$) at 4-cell stage. This correlated with a higher methylation level in the male pronuclei of TX group after fertilization. We also found that at 7, 9 and 11 dpc, the leptin mRNA and the protein were significantly lower ($P<0.05$) in embryos sired by hamsters with all ASG removed compared with the normal control. Our results suggest that the lack of exposure of sperm to paternal accessory sex gland secretions affect the expression of leptin during embryonic development.

This project was supported by a grant (10204227/03678/20200/323/01) from the University of Hong Kong.

Time: 17:30

O17 Histone and protamine compartments of human spermatozoa

A Arpanahi[1], DE Iles[2], M Gilthorpe[4], MH Brinkworth[3], A Balen[1], D Miller[1]

[1]*Centre for Reproduction and Early Development, University Department of Obstetrics and Gynaecology, Leeds General Infirmary, Belmont Grove, Leeds, LS2 9NS, UK*

[2]*School of Biological Science, University of Leeds, Leeds, LS2 9JT, UK*

[3]*School of Biomedical Science, Richmond Building, University of Bradford, Bradford, BD7 1DP, UK*

[4]*Biostatistics Unit, Leeds Institute of Genetics and Health Therapeutics, University of Leeds, Leeds, LS2 9JT, UK*

Introduction: During spermiogenesis, DNA packaging switches from less compact nucleosomal solenoid (histone) bound to more compact torroidal (protamine) bound DNA. This process is incomplete, however, and in humans, ~15% of the DNA remains histone-bound. Previous attempts to characterise this unusual compartmentalisation have proved incomplete and equivocal. Herein we show that permeabilised, salt extracted human spermatozoa are sensitive to endonuclease digestion and that digestion sensitive and insensitive DNAs have markedly different compositions.

Methods: Ejaculate spermatozoa (five normozoospermic samples) were washed free of seminal plasma, permeabilised with CTAB and digitonin and subjected to salt extraction of histones. 'Naked' DNA was recovered and compared with retained (protamine-bound) DNA on Agilent 44B CGH microarrays. Released histones were detected by immunoblotting and CGH data analysed by CGH Analytics and our own bespoke software.

Results & Discussion: Western blot analysis of salt extracts from human spermatozoa showed the presence of acetylated H3 and H4. Data analysis of CGH profiles revealed strong evidence for selective partitioning of gene sequences into nucleosomes over 500 kB intervals. The sex chromosomes, however, were generally histone poor. Nucleosomal domains contained complete, partial and no gene sequences, although limitations in Agilent's probe selection make further interpretation of this finding difficult. We found no evidence linking the selection of genes for nucleosomal packaging with the mRNA composition of ejaculate human spermatozoa. However, the packaging of many important genes in this more open conformation may make them more susceptible to DNA damage and hence lead to potentially higher levels of spermatozoal dysfunction in men exposed to oxidative stressors.

We would like to thank Stephen Krawetz (Wayne State, Detroit, USA) for supplying his mRNA data for comparison.

Time: 17:45

O18 Seminal plasma regulation of cervical cytokine synthesis

Sarah Robertson, David J Sharkey

Research Centre for Reproductive Health, Department of Obsterics & Gynaecology, The University of Adelaide, Adelaide, SA 5005, Austrailia

Introduction: After intercourse, seminal plasma interacts with cervical cells to induce a local inflammatory-like response, influencing the female tract immune response to sperm and sexually transmitted pathogens. This response is characterised by recruitment of inflammatory cells and up-regulated expression of pro-inflammatory cytokines GM-CSF, interleukin-6 (IL-6) and IL-8. Studies in animals have identified TGFβ as the major active constituent in seminal plasma, and TGFβ_1, TGFβ_2 and TGFβ_3 are present in high concentrations in human seminal plasma. The purpose of this study was to investigate whether recombinant TGFβ_1, TGFβ_2, and TGFβ_3 could mimic the effects of seminal plasma on cervical pro-inflammatory cytokine production. The actions of other seminal immune regulators were also examined, including 19-hydroxy prostaglandin E_1 (19-OH PGE_1), and IFNg, identified in mouse studies as being inhibitory to TGFβ actions in uterine epithelial cells.

Methods: Primary cervical epithelial cells prepared from ectocervix of hysterectomy tissues or immortalised Ect1 cells were incubated with recombinant human TGFβ (isoforms 1, 2 or 3), 19-OH PGE_1, or IFNg. Epithelial cell supernatants were analysed by commercial ELISA to quantify GM-CSF, IL-6 and IL-8 production.

Results & Discussion: Each of the three TGFβ isoforms mimicked seminal plasma and were comparable in their capacity to stimulate >5-fold increases in both GM-CSF and IL-6 expression in a dose-responsive manner, while addition of TGFβ neutralising antibodies inhibited seminal plasma-induced increases in these cytokines. Unlike seminal plasma, TGFβ was unable to stimulate IL-8 production. Addition of IFNg was found to strongly inhibit TGFβ-stimulated GM-CSF production, and 19-OH PGE_1 was found to increase IL-6 and IL-8, but not GM-CSF production. These data confirm that TGFβ is the major active constituent in human seminal plasma and indicate that other seminal agents, 19-OH PGE_1 and IFNg, interact with TGFβ to differentially regulate cervical cytokine expression.

FREE ORAL COMMUNICATION SESSION FOUR – OVARY

Date: Tuesday 4 July 2006
Time: 16:30 – 18:00

Time: 16:30

O19 cDNA cloning and expression of the human *NOBOX* gene

John Huntriss, Matthew Hinkins, Helen Picton

Reproduction & Early Development Research Group, University Of Leeds, D Floor Clarendon Wing, Leeds General Infirmary, Leeds, UK

Introduction: *Nobox* is a homeobox gene that is preferentially expressed in the oocyte and is essential for folliculogenesis in the mouse. Nobox is a 'master' transcription factor that regulates the expression of genes essential for oogenesis, including *Dnmt1o, Gdf9, Bmp15, Zar1, Oct4,* and *Mos* among others. Correspondingly, *Nobox -/-* females are infertile. Here we present the first cDNA cloning, characterization and transcript expression analysis of the human *NOBOX* gene. In addition, since a number of additional homeobox genes have expression/functions specifically associated with oogenesis, we have undertaken a detailed study of homeobox gene expression in the human female germline.

Methods: PCR primers designed from the predicted human *NOBOX* sequences were used to characterize putative *NOBOX* transcripts in cDNA libraries generated from staged human ovarian follicles, germinal vesicle-stage oocytes, metaphase II oocytes, preimplantation embryos and somatic tissues. The expression of other homeobox genes was assessed with degenerate *HOX* primers. PCR products were cloned, sequenced and characterized.

Results & Discussion: We have identified and characterized *NOBOX* cDNA clones which span 5 exons. *NOBOX* expression within adult human tissues is limited to the ovary, testis and pancreas. Expression within the ovary is oocyte-specific with expression observed from the primordial stage ovarian follicle through to the metaphase II oocyte. We observed expression of 14 additional homeobox genes throughout human oogenesis and early development. Gastrulation brain homeobox 1 (*GBX1*) and *HOXA7* genes are homeobox markers preferentially expressed by GV oocytes. *HOXA1* and *HEX* are homeobox markers preferentially expressed by metaphase II (MII) oocytes. Homeobox gene transcripts detected in ovarian follicles (*HOXA7, HOXA10, HOXB7, HOXC6, HOXC9, HOXD8*), blastocysts (*HOXB4, CDX2, HOXC9*) and granulosa cells (*HOXA4, HOXA5, HOXA7, HOXC6, HOXC8, HOXC9*) were also identified.

Time: 16:45

O20 CSF candidate Erp1/Emi2 during mouse oocyte maturation

Suzanne Madgwick, Mark Levasseur, Keith Jones

Institute for Cell and Molecular Biosciences, University of Newcastle,Newcastle, NE2 4HH, UK

Introduction: Before fertilisation, vertebrate eggs arrest in metaphase of the second meiotic division (MetII) to await sperm entry due to cytostatic factor (CSF). MetII arrest has been studied extensively in attempts to identify the mechanism of CSF establishment and maintenance. Recently a number of papers identified the protein XErp1/Emi2 as CSF in Xenopus egg homogenates. However XErp1/Emi2 is only one in a long line of potential CSF candidates. With respect to Erp1 it is unclear how well conserved this is, and if Erp1 is involved in establishing MetII arrest as well as maintaining it. An especially important consideration given that establishment of MetII arrest is considered distinct from maintenance of arrest.

Methods: Here we generate and microinject fluorescent protein chimeras to make it possible for us to observe in real time the degradation of Erp1 relative to cyclin B1, a well established marker of APC/C activity and release from MetII arrest. Furthermore, by microinjecting mouse Erp1 morpholinos and fluorescent cyclin B1 cRNA into GV arrested oocytes we can visualise how the absence of Erp1 effects oocyte maturation. Using this method we may pinpoint the stage in the cell cycle when Erp1 becomes an essential part of CSF.

Results & Discussion: We show in intact mouse eggs that mouse Erp1 is extremely stable in MetII arrested eggs and is rapidly degraded on addition of a calcium signal, upstream of the spindle checkpoint apparatus. More importantly, using a mouse Erp1 morpholino, we show that in the absence of Erp1, maturing oocytes undergo chromatin decondensation before they ever form a MetII spindle. The effect of knocking down Erp1 occurs at a much earlier point in the cell cycle than has previously been suggested and demonstrates that Erp1 is essential for the formation of a MetII spindle and the establishment of MetII arrest in mouse eggs.

Time: 17:00

O21 Whole ovary cryopreservation; the effects of Sphingosine-1-Phosphate inclusion

Vicki Onions[1], Marcus Mitchell[1], Bruce Campbell[2], Robert Webb[1]

[1]*School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, Leicestershire, LE12 5RD, UK*

[2]*School of Human Development, University of Nottingham, Nottingham, NG7 2UH, UK*

Introduction: Cryopreservation is hypothesised to result in apoptosis, causing the loss of follicles and damage to stromal tissue following the freeze-thawing of ovarian tissue. The inclusion of anti-apoptotic agents is therefore hoped to improve the efficiency of ovarian cryopreservation, especially in whole ovaries of large animal species where success is limited.

Methods: The ovarian arteries of ovine reproductive tracts were cannulated and the ovary and pedicle dissected free. Each ovary was perfused via the ovarian artery with either a cryoprotectant supplemented with 20μM sphingosine-1-phosphate (S-1-P), an anti-apoptotic agent (n=8), or a non-supplemented control cryoprotectant (n=8), prior to cryopreservation and storage at -196°C. The tissue was then thawed and subjected to a range of viability tests.

Results & Discussion: Granulosa cell viability was not significantly affected by cryopreservation irrespective of treatment (P>0.2); cryopreserved tissue yielded 25.3% (S-1-P) and 24.7% (control) granulosa cell viability compared to 24.7% in fresh tissue. Cellular uptake of BrdU, as a measure of cell proliferation, following culture was seen in both cryopreserved and fresh cortical tissue and 5(6)carboxyfluorescein diacetate succinimidyl ester staining demonstrated many viable small follicles in tissue from all treatments. However S-1-P supplementation did not have any additional effect over the control treatment in terms of granulosa cell survival (P>0.2) or proliferation, as determined by Ki67 expression, (P>0.2).

In conclusion, these results do not support the use of S-1-P at this inclusion level as an anti-apoptotic agent to improve ovarian cell viability following the cryopreservation of whole ovaries. However these results are extremely encouraging for large animal whole ovary cryopreservation, with cell viability being maintained following freeze-thawing.

Time: 17:15

O22 Local regulation of ECM remodelling in the rat ovary

Kirsty Shearer, Micheal Rae, Christopher Harlow, Stephen Hillier

Reproductive and Developmental Sciences, University of Edinburgh, QMRI, 47 Little France Crescent, Edinburgh, EH16 4TJ, UK

Introduction: The extracellular matrix (ECM) is a constituent part of all living tissue, providing structural and physiological support. Within the ovarian follicle, a specialised ECM, the basal lamina, separating the granulosa cells (GC) and theca cells (TC) undergoes continual remodelling during follicle development and ovulation. Three important genes in ECM remodelling are lysyl oxidase (LOX), which cause the cross linkage of collagen and elastin fibrils; bone morphogenetic protein-1 (BMP-1), which proteolytically activates LOX and also cleaves procollagen to collagen; and procollagen C-proteinase enhancer (PCPE), which enhances the activity of BMP-1. We have studied the spatiotemporal expression and regulation of these genes in the follicle wall as a paradigm for generalised tissue remodelling mechanisms.

Methods: GC and TC were isolated from the ovaries of immature rats, some of which received gonadotrophin treatment to induce preovulatory follicular development. RNA was extracted for analysis of target mRNA levels quantitative RT-PCR.

Results & Discussion: By comparing development related mRNA expression profiles of LOX, BMP-1 and PCPE in GC and TC during preovulatory follicular maturation, potential paracrine interactions were deduced. We observed LOX and BMP-1 mRNAs to be more abundant in GC relative to TC throughout antral follicular development. On the other

hand, PCPE mRNA was most strongly expressed in GC and at highest abundance in preantral/early antral follicles. LOX mRNA expression in GC and TC decreased with antral follicular development, while expression of BMP-1 and PCPE were increased relative to LOX throughout. These results suggest that that GC-located LOX rate-limits the degree of collagen cross-linking in the follicle wall, potentially modified by the actions of BMP-1 and PCPE. This indicates potential for gonadotrophin-regulated local interactions between collagen pathway genes in the follicle wall, which are likely to be relevant to the tissue remodelling process elsewhere in the body.

Time: 17:30

O23 Delayed TFAM and PolG activity reduces oocyte competence

Emma Spikings, Justin St. John

The Mitochondrial and Reproductive Genetics Group, University of Birmingham, Birmingham, B15 2TT, UK

Introduction: Cellular ATP is mainly generated through mitochondrial oxidative phosphorylation (OXPHOS), which is dependent on mitochondrial DNA (mtDNA). We have previously demonstrated the importance of oocyte mitochondria for porcine and human fertilisation by assessing oocyte mtDNA copy number, where each mitochondrion possesses one mtDNA molecule. However, the role of nuclear-encoded mitochondrial transcription and replication factors during oocyte development is not yet understood. We have analysed two key factors, mitochondrial transcription factor A (TFAM) and polymerase gamma (PolG), and COX-I, an mtDNA encoded respiratory enzyme, to determine their role in oocyte development.

Methods: Oocyte competence was assessed by glucose-6-phopshate dehydrogenase (G6PD) activity. Developmentally competent oocytes not containing G6PD are unable to break down the Brilliant Cresyl Blue (BCB) dye and stain blue (BCB+). These are more likely to fertilise and have significantly more mtDNA copies than BCB- oocytes. BCB+ and BCB- oocytes were matured in vitro and analysed at the beginning (Day1), middle (Day 2) and end (Day 3) of the maturation process by immunocytochemistry (ICC) and real time RT-PCR.

Results & Discussion: BCB+ oocytes contain high levels of TFAM and PolG transcripts and protein on Day 1. This promotes the increase in COX-I protein seen by Day 3 in these oocytes. After Day 1, TFAM and PolG transcripts and protein decrease to low levels. In contrast, BCB- oocytes contain relatively low levels of TFAM and PolG transcripts and protein on Day 1 and Day 2 and do not increase COX-I protein levels during maturation. However, an increase in both transcripts and protein for TFAM and PolG are observed on Day 3. These results suggest that decreased fertilisation rates for BCB- oocytes could be attributed to delayed mtDNA transcription and replication factor activity during oocyte maturation. This would result in inadequate mtDNA replication prior to fertilisation and insufficient ATP for the maturation process.

Time: 17:45

O24 BMP-6 mediated regulation of BMPR IB expression.

P. Marsters, B. K. Campbell

University of Nottingham, School of Human Development, Division of Obstetrics and Gynaecology, Queen's Medical Centre, Nottingham, NG7 2UH, UK

Introduction: The ubiquitous ovarian, follicular, intercellular, signaller BMP-6 (bone morphogenetic protein-6) is reported to activate signal transduction via BMPR (BMP receptor) dimers consisting of a type I receptor (Activin receptor 1A or BMPR-IB) and a type II receptor (BMPR-II, Activin receptor-II, Activin receptor-IIB). Following the localisation of the FecB (fecundity-enhancing) mutation to the BMPR IB gene it has become of primary importance to clarify the functional involvement of this receptor in intrafollicular cell signalling. Our aims were to investigate the importance of BMPR IB in BMP receptor complexes in ovine ovarian theca cells (TC) and the effect that 'knocking down' its production has on BMP-6 signalling.

Methods: TC were isolated from small antral (1-2mm) ovine follicles and cultured in serum-free media under optimal conditions, to induce differentiation and avoid the problem of spontaneous luteinisation, as previously described (Campbell et al., 1996). Cells were either untreated (NT) or had their production of BMPR IB 'knocked down' utilising siRNA technology under optimal conditions. After 72 hours of culture, media was assayed for androstenedione (A4) by double antibody RIA, while BMPR IB mRNA levels were compared by semi-quantitative RT-PCR using cDNA-recognising primer sets.

Results and Conclusions: BMP-6 addition significantly up regulated BMPR IB mRNA expression ($P<0.001$) and this was abolished in BMPR IB 'knock down' cells. Furthermore thecal cell, A4 production was depressed ($P<0.05$) in BMPR IB 'knock down' cells in the presence and absence of BMP-6. It is concluded that BMP-6 modulates the expression of BMPR IB and that this receptor regulatory cascade is involved in the control of theca cell differentiation.

POSTER ABSTRACTS

P01 Regulation of mitochondrial DNA replication during murine Embryonic Stem Cells differentiation

Joao Facucho-Oliveira, Emma Spikings, Emma Bowles, Jon Alderson, Justin St. John

The Mitochondrial and Reproductive Genetics Group, University of Birmingham, Birmingham, B15 2TT, UK

Introduction: Mitochondria are the eukaryotic organelles responsible for the vast majority of energy production within a cell. This is highly dependent on the process of oxidative phosphorylation (OXPHOS) which in turn requires protein encoded by the mitochondrial genome (mtDNA). Energy deficiencies caused by mitochondrial impairment are closely related to a number of diseases, including neurodegeneration and cardiac dysfunction.
It is therefore important to understand the molecular control of mitochondrial DNA (mtDNA) transcription and replication during the early stages of development. MtDNA replication is regulated by the nuclear encoded mitochondrial transcription factor A (TFAM) and the mitochondrial specific DNA polymerase gamma (PolG). PolG consists of a catalytic subunit (PolG-A) and an accessory subunit (PolG-B) to confer high fidelity DNA polymerisation.

Methods: In this study, we have analysed the expression of TFAM and PolG through relative real time RT-PCR and determined their role in regulating mtDNA copy number during spontaneous differentiation of mouse embryonic stem cells. MtDNA copy number was also measured by real time PCR. Immunocytochemistry (ICC) and confocal microscopy were used to identify the expression of the mtDNA-encoded cytochrome C oxidase I (COXI).

Results & Discussion: Real time PCR revealed that during days 13 and 14 of differentiation, PolGB and TFAM expression was up-regulated by 15% and 20% respectively, while PolGA mRNA levels were found to be similar when compared to the undifferentiated cells. The expression of these transcription and replication factors was found to decrease at days 15 and 16. ICC demonstrated a synchronous increase in mitochondrial content with days 13 and 14. This increase in mtDNA replication factor expression is also reflected in an increase in mtDNA copy number. It is likely that there are key stages in development which are characterised by mtDNA propagation and which may be crucial in determining the final stages of cellular differentiation.

P02 Microarray Analysis of Human Preimplantation Embryo Development

Matthew Hinkins, John Huntriss, Helen Picton

Obstetrics And Gynaecology, University of Leeds, D Floor, Clarendon Wing, Leeds General Infirmary, Leeds, LS2 9NS, UK

Introduction: The study of gene expression in human preimplantation embryos is useful not only for increasing our understanding of early processes in human development such as genomic imprinting, pluripotency and early cell differentiation but also as a tool in the search for potentially reliable genetic markers of embryo quality for use in in vitro fertilization (IVF). Microarrays are a tool that allows the immediate study of the entire transcriptome of a tissue. In this study we have compared the gene expression profiles of individual human embryos against a human PolyA+ reference sample in order to assess gene transcripts specific to preimplantation development.

Methods: Two morulae and five blastocyst stage human preimplantation embryos were collected under ethically approved protocols licensed by the HFEA and lysed in a buffer containing various RNA protectants. mRNA was then extracted from the cells by the use of poly-dT linked magnetic beads and washed. The mRNA transcripts were amplified by the SMART system in order to create a double stranded cDNA library. Two approaches were then compared to label the cDNAs with Cy3 or Cy5 dyes, either a direct labelling process using a klenow fragment or a protocol that enabled the covalent binding of Cy-dyes to the nucleic acid itself. Dye swap reactions with the PolyA+ derived control library were performed. Hybridisations to human genome cDNA arrays were then carried out and transcript expression detected using a Genetic Microsystems 418 scanner and analysed with Arrayvision 8 software.

Results & Discussion: Preliminary results have identified over 200 transcripts that are upregulated in the human blastocyst compared to the PolyA+ sample. Key elements of this data will be discussed in relation to pluripotency and early development in humans. This work was supported by the Birth Defects Foundation.

P03 Genes controlled by two paternally imprinted regions differentially contribute to mouse placentation

Manabu Kawahara, Qiong Wu, Yayoi Obata, Hidehiko Ogawa, Anne C Ferguson-Smith, Tomohiro Kono

Tokyo University Of Agriculture, 1-1-1, Sakuragaoka, Setagaya, Setagay, Tokyo, Japan

Introduction: Imprinted genes have prominent effects on placentation. However, little is known about how genes controlled by two paternally imprinted regions on chromosome 7 and 12 affect mouse placentation. Here, we investigated placentation in ng/fg parthenotes, which contained genomes of non-growing (ng) and fully grown (fg) oocyte through the nuclear transfer technique. In the case of the ng/fg placenta, maternal imprinting was globally modified, and it differed from the imprinting that is observed in case of normal parthenotes; however, paternal imprinting is not imposed. Furthermore, we could understand the role(s) of paternally imprinted genes on chromosome 7 or 12 by application of ng oocytes derived from mutant mice harboring deletion of each paternally imprinted region.

Methods: The genetic backgrounds of ng oocyte were as follows: derived from mutant mice carrying a 13-kb deletion in the *H19* transcription unit with its differentially methylated region (*H19*-DMR) on chromosome 7 ($ng^{\Delta ch7}$); and from mutant mice carrying a 4.15–kb deletion in the intergenic germline-derived DMR (IG-DMR) on chromosome 12 ($ng^{\Delta ch12}$). Besides, ng oocytes of wild type (ng^{WT}) were used for oocytes reconstruction. After preparing ng/fg placentae, we carried out the following analysis: quantitative analysis of gene expression, mRNA in situ hybridization, histological and morphometrical analysis and SEM studies of corrosion casts.

Results & Discussion: We confirmed by gene expression analysis that the expression of *Igf2* and *Dlk1* gene were corrected in $ng^{\Delta ch7}$/fg and $ng^{\Delta ch12}$/fg placentae, respectively. Furthermore, we showed that $ng^{\Delta ch7}$/fg placentae increased the whole mass with morphologically normal giant cells, whereas $ng^{\Delta ch12}$/fg placentae were extremely small with the orderly three layers. Our findings demonstrate that genes controlled by two paternally imprinted regions, *H19*-DMR or IG-DMR, differentially contribute to mouse placentation.

P04 Effect of timing and amount of GM-CSF on IVC of bovine embryos

Cristina Fontes Lindemann, Alison Ainslie, Cheryl Ashworth, John Rooke

Scottish Agricultural College, West Mains Road, Edinburgh, UK

Introduction: Inclusion of Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF) in embryo culture media inhibits apoptosis and promotes blastomere proliferation, viability and differentiation in human and murine embryos. However, effects of GM-CSF are less well established in ruminants. The objective of this study was therefore to determine the optimum timing and concentration of GM-CSF during in vitro culture (IVC) of bovine embryos.

Methods: Oocytes were aspirated from abattoir-derived ovaries and underwent in vitro maturation, fertilization and culture in modified synthetic oviductal fluid (SOF) containing bovine serum albumin (3mg/mL) and non-essential amino acids (1% v/v). Cleaved embryos were selected 32h post-fertilisation (on day 1) and assigned (groups of 10 in 0.05mL drops) to SOF with or without recombinant ovine (ro) GM-CSF. Embryos were transferred to fresh media every 48h. Blastocyst development, morphology and diameter were evaluated on days 7, 8 and 9. Day 7 and 8 blastocysts were cultured for a further 24h in individual 0.05ml microdrops of SOF and total and apoptotic (TUNEL assay) cell numbers recorded. In experiment 1, embryos were cultured in 0, 2, 5, 10 or 50ng/mL roGM-CSF for 48h from days 1 to 3 and then transferred to SOF without roGM-CSF. In experiment 2, 2 or 10ng/mL GM-CSF was absent(A) or present(P) between days 1 and 3 only (PA) or throughout culture (PP, days 1 to 9) giving five combinations: AA, 2PA, 10PA, 2PP and 10PP.

Results & Discussion: Blastocyst yields were 35% (from 730 cleaved zygotes), and 20% (from 795 cleaved zygotes) in experiments 1 and 2 respectively. Timing of exposure and amount of roGM-CSF had no significant effect on overall blastocyst yield or quality. Bovine embryos were therefore unresponsive to roGM-CSF in vitro at the concentrations and developmental periods tested. SAC receives financial assistance from Scottish Executive Environment and Rural Affairs Department.

P05 NOS expression in the mouse preimplantation embryo

Paula Jane Stokes, Frachesca Dawn Houghton

Human Genetics Division, Duthie Building, Mailpoint 808, University of Southampton, Southampton General Hospital, Tremona Road, Southampton, SO16 6YD, UK

Introduction: Nitric oxide (NO) production plays an important role in regulating mouse preimplantation embryo development. NO is produced from l-arginine by the enzyme nitric oxide synthase (NOS), which has three isoforms: endothelial (eNOS), inducible (iNOS), and neuronal (nNOS).

Methods: The localisations of these proteins and the effect of amino acid supplementation to KSOM culture medium on their expression in preimplatation mouse embryos were determined by immunocytochemistry and confocal microscopy. nNOS was not observed, however, eNOS and iNOS were present throughout development. The proteins were predominantly located to the apical periphery of blastomeres prior to cavitation, and to a lesser extent in the nucleus. At the blastocyst stage iNOS and eNOS protein expression was significantly reduced in the inner cell mass compared to the trophectoderm.

Results & Discussion: In the presence of amino acids iNOS expression was not affected, however, eNOS expression was significantly higher at all stages of preimplantation development. Since NO is produced from l-arginine, it can be speculated that an increase in eNOS is a cellular response to the increase in intracellular arginine, thus increasing NO production by the embryo. Indeed, the removal of arginine from KSOMaa medium resulted in no increase in eNOS expression compared to those cultured without amino acids.

P06 Antioxidants for the cryopreservation of bovine oocytes

Sarah Harris[1], Adam Glaser[2], Vinay Sharma[2], Tony J Rutherford[3], Helen M Picton[1]

[1]*Obstetrics And Gynaecology, University of Leeds, D Floor, Clarendon Wing, Leeds General Infirmary, Leeds, LS2 9NS, UK*

[2]*St. James Hospital, Beckett Street, Leeds, LS9 7TF, UK*

[3]*Leeds General Infirmary, Great George St, Leeds, LS1 3EX, UK*

Introduction: Cryopreserved oocytes experience oxidative stress during cooling and warming. This study supplemented cooling and warming solutions for vitrification and slow-freezing of immature (GV) and matured (MII) bovine oocytes with the antioxidants pyruvate, ascorbate or EDTA.

Methods: Oocyte-cumulus complexes (OCCs) were vitrified in open-pulled straws (OPS) or slow-cooled in straws either before or after maturation (IVM) in serum-free medium. Serum-free HMEM-based cooling and warming solutions for slow-freezing (1.5M PROH, 0.3M sucrose) or vitrification (20% DMSO, 20% EG, 0.6M sucrose) were supplemented with 100μM ascorbate, 10μM EDTA, or 0.5mM pyruvate. Post-thaw, oocyte survival and developmental competence was assessed by morphology, Neutral Red staining, Orcein staining, Zona Pellucida (ZP) hardening, IVM, IVF and embryo development.

Results & Discussion: 1117 OCCs were analysed. Vitrification yielded better survival rates than slow-freezing, regardless of oocyte maturity or supplementation. <10% of GV-OCCs survived slow-freezing. Pyruvate and EDTA significantly increased survival of slow-frozen MII-oocytes (57% and 54%, respectively) compared to ascorbate (35%) or controls (33%). Survival of vitrified GV-OCCs was significantly higher after supplementation with pyruvate (83%) or EDTA (74%) compared to controls (53%). Survival of vitrified MII oocytes was approximately 78%. All cryopreserved oocytes underwent ZP hardening. No slow-frozen GV-oocytes resumed nuclear maturation. In contrast, 92% of vitrified GV-oocytes resumed meiosis. Cryopreservation supplements did not affect fertilisation or embryo development. Only 7% of slow-frozen MII-oocytes cleaved, whereas 34% of GV and 14% of MII vitrified oocytes progressed to cleavage stages. In conclusion, these data suggest that vitrification supports high rates of oocyte survival and developmental competence in bovine OCCs compared to slow-freezing.

P07 Oocyte retrieval in superstimulated buffalo heifers

Gianluca Neglia, Mariafrancesca Maschio, Simona Allegrini, Marco Mazzi, Antonio Borghese

Istituto Sperimentale per la Zootecnia, Via Salaria 31,Monterotondo Scalo -Roma, Italy

Introduction: Embryo production following oocytes in vitro fertilization is related on number of oocytes collected by Ovum pick-up. The objective of this study was to compare stimulated peripuberal buffaloes and to assess ovarian response and oocyte recovery.

Methods: On day 0, twelve heifers undergone clinical examination of genital tract by ultrasound and a progesterone releasing intravaginal device (PRID®, Vetem, Italy) was inserted into the animals. On day 3 buffaloes were divided into two treatment groups, according to their age, body weight and follicular population on day 0 and 3. Heifers in Group A (n=6) received a 4-day decreasing dosage of an equal mixture of 500 I.U. of Follicle Simulating Hormone and 500 I.U. of Luteinizing Hormone (Pluset, Serono Veterinary, Italy); Group B (n=6) received a single i.m. injection of Pregnant Mare Serum Gonadotrophin (PMSG, Folligon, Intervet, Italy) and a 2-day decreasing dosage of an equal mixture of 175 I.U. of FSH and 175 I.U. of LH, starting on day 5. On day 7 all buffaloes undergone ultrasound examination and number and size of the follicles was recorded.

Results & Discussion: Group B resulted in a significantly higher ($P<0.05$) number of large-very large follicles compared to group A (13.2 vs 8.3). Although no significant differences, in group A and B, were found on the number of observed follicles (14.2 vs 18.2), aspirated follicles (11.5 vs 14.0), recovered oocytes (6.33 vs 6.5) and percentage of recovered oocytes/ aspirated follicles (60.0 vs 44.8), all the parameters tended to be higher in group A. The percentage of viable oocytes (3.2 vs 3.2) was similar. Protocol B stimulated the recruitment and the growth of a higher number of small follicles. It is possible to hypothise that the higher size of the follicles in group B, caused the lost of follicular fluid affecting oocyte recovery.

P08 Porcine oocyte secreted protein analysis

Bob Robinson, Susan Liddell, Bob Webb, Morag Hunter

Animal Physiology, Sutton Bonington Campus, University of Nottingham, Loughborough, LE12 5RD, UK

Introduction: We have previously shown that oocyte conditioned medium (OCM) or co-culture with oocytes at the germinal vesicle (GV), but not metaphase II (MII) stage modifies granulosa cell steroidogenesis and proliferation. Thus the objective of this study was to investigate de novo synthesised and secreted proteins by porcine oocytes at these two stages of development.

Methods: In Expt 1, 200 porcine GV oocytes were labelled with 200 μCi ^{35}S-methionine. After 4 h, oocytes and OCM were collected and run on 12% SDS-PAGE. In Expt 2, porcine oocytes were collected at either the GV (n=1700) or MII (n=1700) stage. The MII oocytes were matured in M199 supplemented with 200ng/ml LH, 50ng/ml FSH and 10ng/ml EGF for 44h. The GV and MII oocytes were denuded, washed and cultured in M199 containing 0.1% PVP for 4h prior to OCM collection. The samples were prepared by buffer exchange on YM-10 filter followed by precipitation (GE Healthcare, 2D Clean-Up Kit). Proteins were focussed on 7cm non-linear IPG strips pH3-10 (Bio-Rad), separated by 12% SDS-PAGE and then stained with silver nitrate.

Results & Discussion: In Expt 1, one-dimensional analysis of newly synthesised GV OCM revealed the presence of 7 major bands at approximately 10, 20, 25, 40, 50, 60 and 80 kDa. In GV oocytes, bands at similar weights, plus an additional 5 bands were detected. In Expt 2, 2-D gel analysis of GV OCM (approximately 1μg protein) revealed 23 protein spots. The majority of these spots were either absent or of lower intensity in MII OCM. In conclusion, we have shown that denuded porcine oocytes synthesised proteins *de novo* and that a number of these proteins appear to be secreted. Furthermore, differences were detected in the secreted proteome from GV and MII oocytes. Future work will be directed towards the identification of these secreted proteins.

P09 Lipid:mitochondrial FRET in mammalian eggs

Roger G. Sturmey[1], Peter O'Toole[2], Henry Leese[1]

[1]*Department of Biology (Area 3), Univeristy of York, Heslington, York, YO10 5YW, UK*

[2]*Technology Facility, University of York, Heslington, York, YO10 5YW, UK*

Introduction: Porcine oocytes and early embryos are characterized by a large amount of intracellular lipid, consisting mainly of Triglyceride (TG). There is evidence that this intracellular TG is metabolised to generate ATP during egg *in vitro*-maturation. TG is metabolised by β-oxidation and TCA cycle, processes occurring within the matrix of mitochondria and re-location of mitochondria has been reported during the maturation of mammalian oocytes in a variety of species.

Methods: Laser Scanning Confocal Microscopy (LSCM) and Fluoresence Resonance Energy Transfer (FRET) were used to examine lipid:mitochondrial co-localisation in slaughterhouse derived porcine oocytes, stained with Mitotracker Green (MTG) (mitochondria) and Nile Red (NR) (lipid droplets). Dual labelled images were Spectrally Unmixed using spectral profiles obtained from single stained samples on a Zeiss LSM 510 META inverted confocal microscope. We asked 1) whether there was sufficient overlap between the emission of Mitotracker green and Nile Red excitation to support a FRET reaction and 2) if mitochondria and lipid droplets were sufficiently co-localised to FRET. NR was specifically bleached using a HeNe 543 laser . FRET experiments were performed on four separate occasions with 11 experiments carried out in total.

Results & Discussion: Bleaching of the NR acceptor molecule lead to a visible increase in the MTG signal, equivalent to a mean FRET efficiency of 22.2 +/- 3.18 %. These data provide the strong evidence that MTG and NR can act as a FRET pair and also offers proof in live cells (i.e. the oocyte) that mitochondria and lipid droplets lie within 10nm, most likely closer, of each other, indicating true co-localisation on a molecular scale. Since TG is metabolised within the mitochondrial matrix, it is appropriate that these organelles be spatially associated to facilitate the rapid transport of the free fatty acid molecules to the site of metabolism.

Funding; BBSRC & Sygen International .

P10 Developmental potential of pig oocytes cultured at low physiological temperatures and fertilised in vitro: effect of culture media

Jinpei Ye, Keith Campbell, Martin Luck

Division of Animal Physiology, University of Nottingham, Sutton Bonington Campus, Loughborough, LE12 5RD, UK

Introduction: Low physiological temperatures slow but support nuclear maturation in pig oocytes. However the quality of such matured oocytes is unknown. We examined the embryo development of oocytes cultured at reduced temperature in different media and fertilised *in vitro*.

Methods: Cumulus enclosed oocytes were preincubated with cycloheximide (CHX, 16h) at 39, 37 or 35.5°C. They were then cultured without CHX for varied periods, either at the same temperature or continuing at 39°C for 8h followed by culture at 37 or 35.5°C for 16h. The culture media were defined medium (DM; M199 containing 0.1% BSA, 0.57 mM cysteine, 0.2 μg/ml LH, 10 ng/ml EGF), DM plus 50 ng/ml FSH (FDM) and FDM plus 20% porcine follicular fluid. *In vitro* fertilisation (IVF) and embryo culture were performed at 39°C. A total of 3506 oocytes, with three batches for each treatment were studied and analysed (ANOVA; $p<0.05$ for significance).

Results & Discussion: Although similar proportions of oocytes cleaved (> 50%) after IVF, the rates of blastocyst formation were significantly lower from oocytes cultured with DM at 35.5°C for 60h (2.6+/-1.4%), 37°C for 36~48h (4.4+/-2.2%~2.8+/-1.9%) and 39°C for 36h (4.2+/-2.7%) than at 39°C for 24h (19.6+/-3.1%). Culture at 35.5°C for 36h significantly decreased the rate of cleavage (23.4+/-4.8%). However, rates of cleavage and blastocyst formation from oocytes cultured at 35.5°C or 37°C for 16h after an initial 8h of culture at 39°C were similar to those from oocytes cultured at 39°C for 24h. Culture with FSH increased blastocyst yields at 37°C and 39°C but had no effect at 35.5°C. However, culture with follicular fluid significantly increased rates of cleavage (50.2+/-5.2%) and blastocyst formation (13.8+/-1.4%) at 35.5°C. In conclusion, culture at 35.5°C compromises the developmental potential of pig oocytes, but follicular fluid may offer protection against the detrimental effects of low temperatures.

Supported by BBSRC.

P11 Real time RT-PCR analysis of heat shock protein 70 transcripts (mRNA) in pig oocytes cultured at a range of physiological temperatures

Jinpei Ye[1], Tim Parr[2], Keith Campbell[1], Martin Luck[1]

[1]*Division of Animal Physiology, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, LE12 5RD, UK*

[2]*Division of Nutritional Sciences, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, LE12 5RD, UK*

Introduction: Low physiological temperatures slow nuclear maturation in pig oocytes. Fertilisation remains possible but a culture temperature as low as 35.5°C may compromise oocyte developmental potential. To reveal the mechanism of the action of temperatures, we have analysed heat shock protein (HSP) 70 mRNA levels in these oocytes.

Methods: Cumulus enclosed oocytes were synchronised by preincubation with cycloheximide (5μg/ml, 16h) and then cultured in medium199 (containing 0.1% BSA, 0.57 mM cysteine, 0.2 g/ml LH and 10 ng/ml EGF) over a time course at 39, 37 or 35.5°C. Meiotic progression was monitored. Thirty oocytes were pooled at each time-point and denuded. At each time-point total RNA was extracted with a RNeasy Micro kit (Qiagen) and first strand cDNA synthesised from 4.56ng total RNA using a Sensiscript RT kit (Qiagen) and random hexamers. HSP70 and β-actin mRNA levels were quantified by Quantitative PCR using porcine specific primers and probes (HSP70 F/R: 5'-ATCCCCACCAAGCAGACG-3'/5'-CAGGTTGTTGTCCCGCGT-3', probe 5'-FAM-CGTACTCGGACAACCAGCCGGG-TAMARA-3'; β-actin F/R: 5'-CTTCCAGCCCTCCTTCCTG-3'/5'-CGTAGAGGTCCTTGCGGATG-3', probe 5'-FAM-AATCCTGCGGCATCCACGAAACTACC-TAMARA-3'). Transcript levels were expressed equivalent total RNA. Comparisons were made using three batches of oocytes for each time-point (ANOVA; $p<0.05$ for significance).

Results & Discussion: There was no significant difference in β-actin transcript levels in oocytes cultured for 0, 4, 6, 8, 24, 36 and 60h at 39, 37 and 35.5°C. As a ratio of HSP70 to β-actin, the levels of HSP70 transcript were also similar at 39°C (0.76) and 37°C (0.70) over culture time, but significantly higher at 35.5°C (0.84), declining significantly after 60h of culture (0.54). Consistently, the ratios were significantly higher at 35.5°C during GV-GVBD (0.86) but lower at MII (0.54) than at 39 (0.72 and 0.78) and 37°C (0.72 and 0.83). In conclusion, the expression profile of HSP70 transcript in oocytes culture at 35.5°C appeared to be different from those at 39 and 37°C.

Supported by BBSRC.

P12 Gonadotrophin-dependent modulation of granulosal and thecal expression of activin receptors and inhibin co-receptor in chicken prehierarchical and preovulatory follicles.

Tristan Lovell, Sara Al-Musawi, Richard Gladwell, Philip Knight

School of Biological Sciences, University of Reading, Whiteknights, Reading RG6 6AJ, UK

Introduction: Ovarian follicle development is regulated through both endorine and local control mechanisms with increasing evidence indicating a role for inhibins and activins. We recently indentified divergent *in vivo* expression of mRNAs encoding activin receptors (ActR) and inhibin co-receptor betaglycan in avian ovarian follicles. In this study we used quantitative(Q)-PCR to compare the actions of LH and FSH (0, 1, 10, 100ng/ml) on *in vitro* expression of ActRI, ActRIIA, ActRIIB and betaglycan mRNAs in isolated chicken granulosa (GC) and thecal (TC) cells from the F1 preovulatory and 6-8mm prehierarchical follicles.

Methods: RNA was purified (following 24h plating and 2x 24h treatment) from cultured 6-8mm and F1 GC and TC cells and cDNA synthesized. QPCR reactions were carried out using primers/probes designed to target mRNA. Receptor expression data were normalized to GAPDH.

Results & Discussion: FSH promoted a significant decrease in ActRI and ActRIIB expression in 6-8mm GC whereas LH caused a significant increase. Both LH and FSH enhanced ActRIIA (5-fold and 8.5-fold) and betaglycan expression (2-fold and 3.5-fold) in 6-8mm GC. In 6-8mm TC, LH and FSH both increased betaglycan expression (7-fold and 3.5-fold) but did not affect ActRI, ActRIIA and ActRIIB expression. In F1 GC both LH and FSH stimulated ActRI (2-fold and 2.4-fold), ActRIIB (3.2-fold and 2.7-fold) and betaglycan (7-fold and 4-fold) while ActRIIA was unaffected. In F1 TC, LH and FSH reduced ActRIIA (35 50%) and increased betaglycan expression (4.5-fold and 7.6-fold), but had no effect on ActRI and ActRIIB expression. These results indicate that follicular expression of activin receptors and betaglycan is differentially regulated by gonadotrophins during follicle maturation in the hen. This may represent an important mechanism for fine-tuning follicle responsiveness to local and systemic levels of activin and inhibin, thereby modulating follicle development and progression into and through the preovulatory hierarchy.

Supported by BBSRC.

P13 Measurement of glucose and lactate metabolism of isolated ovine follicles in vitro

Rosa Garcia[1], Sarah Harris[2], Ping Jin[2], Helen Picton[2]

[1]*Facultad De Veterinaria, Universidad Complutense de Madrid, Madrid, 28040, Spain*

[2]*Obstetrics And Gynaecology, University of Leeds, D Floor, Clarendon Wing, Leeds General Infirmary, Leeds, LS2 9NS, UK*

Introduction: The progression of follicular metabolism during ovine folliculogenesis has not been exhaustively investigated. The aim of present study was to examine glucose and lactate metabolism in isolated ovine follicles from primary to early antral stages of development.

Methods: A total of 151 follicles were isolated by enzymatic (n=84) or mechanical (n=67) methods from fresh ovarian tissue. Follicles were allocated into 4 groups: enzymatically-isolated (EI) follicles cultured for 2 or 3 days and mechanically-isolated (MI) follicles cultured for 2 or 3 days. Theca-free follicles were enzymatically-isolated from ovarian cortex using a collagenase-DNase enzyme mixture. Theca-intact follicles were mechanically-harvested under a dissecting microscope using a scalpel blade. Individual follicles were cultured in serum-free alpha-MEM-based medium at 39°C in 5%CO_2 according to the method of Newton et al., 1999 (J.Reprod.Fert. 115:141-150). At the end of culture, spent media were analysed for glucose and lactate concentrations using a colourimetric assay.

Results & Discussion: Overall, two day cultures showed a significantly lower glucose: lactate ratio than three day cultures (0.42±0.06 vs 0.85±0.09, P=0.0000). There was no significant difference in glucose: lactate ratio of EI2 and MI2 follicles (0.47±0.11 and 0.63±0.11, P=0.168). In contrast, glucose: lactate ratios of EI3 and MI3 follicles were 0.62±0.11 and 0.36±0.11, P=0.0318). Furthermore, larger follicles consistently showed a significantly higher glycolytic index compared to smaller follicles, with 13-25% glycolytic glucose consumption by 231-500μm EI2/EI3 follicles compared to 100% glycolytic glucose consumption by 701-1000μm EI2/EI3 follicles. Similarly, glycolytic glucose consumption was 18-25% by 231-500μm MI2/MI3 follicles compared to 38-70% glycolytic glucose consumption by 701-1000μm MI2/MI3 follicles. In conclusion, theca-free enzymatically-isolated follicles were more dependent on glycolytic glucose metabolism than theca-intact mechanically-isolated follicles over 3 days *in vitro*. An increased glycolytic index was associated with more advanced follicles and was elevated after enzymatic isolation.

P14 Direct *in vitro* evidence that the anti-epileptic drug valproic acid suppresses rather than enhances thecal androgen output

Claire Glister, Katherine Briley, Emma Perks, Phil G Knight

School Of Biological Sciences, University of Reading, Whiteknights PO Box 228, Reading, RG6 6AJ, UK

Introduction: Valproic acid (VPA) has been commonly used to treat epilepsy for over 30 years. Women undergoing VPA treatment reportedly have an increased incidence of a polycystic ovarian (PCO)-like syndrome, characterized by weight gain, hyperandrogenemia and hyperinsulinemia. A recent *in vitro* study involving 'passaged' human theca cells (TC) indicated a direct stimulatory action of VPA on androgen output. This prompted us to investigate the effect of VPA using primary cultures of bovine theca (TC) and granulosa cells (GC) maintained under well-defined, serum-free conditions in which these cells retain their follicular phenotype.

Methods: Mural GC and TC were retrieved from 4-6mm follicles and cultured for 144h with media changes every 48h. Viable cell number was determined at the end of culture. Effects of VPA (7.8-500 μg/ml) on TC were tested alone and in combination with LH (4-2500 pg/ml). Effects of VPA on GC were tested alone and in combination with FSH (0.037-3 ng/ml) or LR3IGF-1 (4-100 ng/ml). Results are based on combined data from >4 independent cultures and are presented for the last time period (96-144h).

Results & Discussion: Contrary to findings in human TC, VPA dose-dependently reduced both basal (70% suppression; P<0.001) and LH-induced androgen secretion (up to 93% suppression; P<0.0001) by bovine TC. VPA did not affect basal progesterone secretion by TC, but had a slight

suppressive effect on progesterone secretion induced by the highest LH-dose tested; TC number was unaffected by VPA. At higher doses (125-500 μg/ml) VPA inhibited basal, FSH- and IGF-stimulated oestrogen secretion ($P<0.0001$) but did not affect progesterone secretion or cell number. In conclusion, these findings firmly refute the hypothesis that VPA has a direct stimulatory action on TC androgen output. On the contrary, VPA clearly inhibits LH-dependent androgen production in this bovine model.

Supported by BBSRC.

P15 Fertility of Mice Receiving Vitrified Adult Mouse Ovaries

Toshio Hani, Takanori Tachibe, Saburo Shingai, Nobuo Kamada, Otoya Ueda, Kou-ichi Jishage

Chugai Research Institute for Medical Science, 1-135 Komakado, Gotemba Shizuoka, Japan, 412-8513

Introduction: Cryopreservation of the ovaries is a useful technology for preservation of germ cells from experimental animals, because if the female founder is infertile or has mutated mitochondrial DNA, preservation of female germ cells is necessary. Although it is possible to cryopreserve immature mouse ovaries with a high degree of viability using vitrification with a mixture of several cryoprotectants, the viability of cryopreserved adult mouse ovaries is still unknown. Here, we investigated the viability of mouse ovaries at various ages after cryopreservation using vitrification techniques.

Methods: Donor ovaries were collected from 10-week- and 7-month-old GFP-transgenic mice (genetic background: C57BL/6J(B6)). The isolated ovaries were cut into small pieces of about 1 mm^3 (donor ovary) in cooled Whitten's medium. Then they were frozen-thawed by vitrification as described by Migishima. The frozen-thawed ovaries were orthotopically transplanted to 4-week- and 10-week-old B6 mice (recipients). Recipients were mated with B6 male mice 3 weeks after ovary transplantation. The pups from donor ovaries showed green fluorescence under UV light.

Results & Discussion: GFP-positive pups were obtained in all experimental groups. In the 4-week-old recipients, the percentages of GFP-positive pups among the total pups from recipients transplanted with ovaries of 10-day-, 4-week-, 10-week-, and 7-month-old donors were 44%, 9%, 12%, and 4%, respectively. In the 10-week-old recipients, the percentages of GFP-positive pups among the total pups from recipients transplanted with ovaries of 10-day-, 4-week-, 10-week-, and 7-month-old donors were 36%, 16%, 2%, and 9%, respectively. Furthermore, GFP-positive pups also were obtained from recipients transplanted with ovaries of donors without normal estrus cyclicity. Our results indicate that cryopreservation of mouse ovaries by vitrification is a useful method for the preservation of female germ cells from mice of various ages.

P16 Monosaccharide utilization by cultured ovine granulosa cells

Nigel R Kendall[1], Rex J Scaramuzzi[2], Bruce K Campbell[1]

[1]*Obstetrics And Gynaecology, School Of Human Dvelopment, University of Nottingham, Queen's Medical Centre, Nottingham, NG7 2UH, UK*

[2]*Department of Veterinary Basic Sciences, Royal Veterinary College, London, UK*

Introduction: Glucose is the major energy source utilised by man and animals. However, other monosaccharides are also available in the diet. Fructose is available from fruit, honey, high fructose corn syrup and the digestion of sucrose and galactose is a constituent of the milk sugar lactose and is in plentiful supply in the modern diet.

Methods: Ovine ovaries from an abattoir were used to provide granulosa cells from 1-3 mm follicles. Granulosa cells were plated at 75 x 10^3 cells per well in a 96 well plate and cultured for 192h in custom made serum and glucose free media with 3g/l of glucose, galactose or fructose. Oestradiol production was measured by radioimmunoassay, cell number by neutral red and lactate production by colourimetry. Data were analysed after appropriate transformation, by ANOVA using SPSS 14.0.1

Results & Discussion: Oestradiol production was significantly greater ($P<0.001$) for the glucose (320 pg/48h) than for galactose (223 pg/48h) or fructose (207 pg/48h). Cell number at the end of the culture (192h) was significantly greater ($P<0.001$) for the glucose (43.3 kcells) than for galactose (28.6 kcells) or fructose (36.6 kcells). Oestradiol production per 10^3 cells at 192 h was significantly greater ($P<0.05$) for the glucose (11.3 pg/kcell) than for galactose (8.8 pg/kcell) or fructose (6.5 pg/kcell). Lactate production was significantly higher ($P<0.01$) for glucose (11.5 mg/dl) than fructose (1.6 mg/dl) or galactose (0.9 mg/dl). There were no significant differences between fructose and galactose for any of these parameters. Glucose was the superior energy source for the cultured ovine granulosa cells. However, both fructose and galactose were able to support cell survival and oestradiol production. Glucose was the only treatment that resulted in a significant production of lactate, indicating that granulosa cells may not be able to metabolise fructose and galactose using anaerobic pathways.

P17 Follicular dynamics in oestrus synchronized buffaloes

Corrado Pacelli[1], Mariafrancesca Maschio[2], Giuseppe De Santis[2], Simona Allegrini[2], Giuseppina Maria Terzano[2], Vittoria Lucia Barile[2]

[1]*Dipartimento di Scienze delle Produzioni Animali, Università della Basilicata, Potenza, Italy*

[2]*Istituto Sperimentale per la Zootecnia, Monterotondo-Roma, Italy*

Introduction: In the last years, ultrasound monitoring of ovarian structures in buffalo has provided the basis for improving fertility and applying biotechnology, but little has been published on the follicular dynamics during the induced oestrus. Thus, the purpose of the present work was to study follicular dynamics in buffalo cows, treated with two different hormonal protocols for oestrus synchronization in two different period of the year in order to evaluate their efficiency to apply fixed time artificial insemination.

Methods: Twenty-four lactating buffaloes, were equally assigned to two different oestrus synchronization treatments, PRID associated with PMSG and $PGF_{2\alpha}$ or Ovsynch, in two different period of the year: February (end of the natural breeding period) and May (beginning of the non-breeding period). Ovarian ultrasound examination was performed during the treatment period and until ovulation occurred by a 7.5 MHz linear rectal probe. Data were analyzed by ANOVA and x^2 test.

Results & Discussion: The mean size of dominant follicle was not affected neither by the synchronization treatment or by the period in witch the treatment was effected. The dominant follicle was bigger ($P<0.05$) in buffaloes treated with PRID compared with those treated with Ovsynch (cm 1.54±0.09 vs 1.29±0.08) in the preovulatory phase (36 h before ovulation). The size of preovulatory follicle was larger in animals treated in February compared with those treated in May (cm 1.51±0.07 vs 1.32±0.07 respectively). A good ovulation rate was obtained in both treatments (100% with PRID and 75.0% with Ovsynch), while a higher ovulation synchronization rate ($P<0.07$) was found in the Ovsynch protocol compared with the PRID one (100% vs 66.6%). The rate of ovulated animals was not affected by the month of treatment.

P18 Ghrelin in the chicken ovary: expression, effects and mechanisms of action

Alexander Sirotkin[1], Roland Grossmann[2], Manuel Tena-Sempere[3]

[1]*Dept. Animal Genetics & Reproduction, Research Institute of Animal Production, Slovak Centre of Agricultural Studies, 949 92 Nitra, Slovakia*

[2]*Dept. Functional Genomics & Bioregulation, Institute of Animal Science, Federal Research Centre, Mariensee, 31535 Neustadt, Germany*

[3]*Department of Cell Biology, Physiology and Immunology, University of Cordoba, 14004 Cordoba, Spain*

Introduction: The role of the newly discovered anorexic peptide ghrelin (G), consisting of 28 amino acids, in controlling reproduction remains to be studied. The aim of this study, performed on chicken, was to evaluate the expression of G and its receptor (GHS-R) genes in the ovary, to examine the direct effect of G, its analogues and molecular fragments on ovarian cell function, and to outline intracellular protein kinases mediating these effects.

Methods: Fragments of chicken ovarian follicles or ovarian granulosa cells were cultured in the presence of G, synthetic activator of its receptor (GHS) and molecular fragments of G (G1-18 and G 1-5; 1, 10 or 100 ng/ml). The presence of G and GHS-R1a mRNAs in the ovary were analysed by RT-PCR. Accumulation of proliferation- and apoptosis-related peptides and some protein kinases were evaluated using immunocytochemistry, Western immunoblotting and TUNEL, while secretion of hormones was measured by RIA.

Results & Discussion: Our results demonstrate that both ghrelin and GHS-R1a mRNAs are expressed in chicken ovarian tissue. Moreover, challenge of ovarian cells with G or G 1-18, but not G1-5, was able to induce markers of proliferation (i.e., expression of both PCNA and cyclin), to decrease the expression of markers of apoptosis (caspase-3, bax, bcl-2, p53 and TUNEL-positive cells) and to stimulate the release of progesterone, oestradiol, arginine-vasotocin and IGF-I, but not testosterone. These changes were associated with accumulation of protein kinase A (PKA), MAP kinase (MAPK) and tyrosine kinase within ovarian cells. Blockers of PKA, MAPK and CDC2 kinase were able to promote or prevent some effects of G1-18. Our study provides new evidence for the gonadal expression of genes encoding G and its cognate receptor in the chicken ovary and unravels the potential involvement of this newly discovered peptide in the control of key gonadal functions in the chicken, such as proliferation, apoptosis, and hormone release. The part of the G molecule corresponding to amino acids 5-18, but not to amino acids 1-5 is responsible for these effects. The effects of G and its analogues are mediated by GHS receptors and by PKA-, MAPK-, tyrosine kinase and CDC2 kinase-dependent intracellular mechanisms.

P19 Effect of antiestrogens on proliferation and telomerase activity of pig granulosa cells in vitro

Milan Tomanek, Ewa Chronowska, Tomas Kott

Research Institute Of Animal Production, Pratelstvi 815, P.O.Box 1, Prague 10 - Uhrineves, Prague, CZ 104 01, Czechoslovakia

Introduction: Estrogens are acknowledged as potent regulators of ovarian follicle development. Expression of telomerase (TERT) was determined in ovary and it is hypothesized that telomerase activity (TA) is under hormonal control in some estrogen-targeted tissues including the ovary. Estrogen receptor (ER) alfa and beta are expressed in the pig ovary and recently we confirmed the TA in pig granulosa cells. This study was conducted to assess the effect of ER antagonists on pig granulosa cell proliferation and TA *in vitro.*

Methods: Granulosa cells (GC) from small (1-2mm, SF) and large (6-7mm, LF) follicles were cultured in vitro in DMEM/F12 medium supplemented with 2% FBS, ITS, gentamycin and 10^{-8} M of testosterone. Antiestrogen ICI 164.384 and ERβ selective modulator cyclofenil were used in a concentration of 10^{-7} M. EGF (10ng/ml) and pFSH (50ng/ml) were used to stimulate the cells. Proliferation of GC was measured by ^{3}H-thymidine incorporation and LSC counting. Telomerase activity was assayed using the modified protocol based on the TRAPEZE® Telomerase Detection Kit. FAM labeled PCR product was analyzed with sequencer.

Results & Discussion: Antiestrogens decreased proliferation of SF and LF-GC differentially. No significant inhibitory effect of cyclofenil was observed in non-stimulated SF-GC. Significant decrease (48-50%) of proliferation was found in LF-GC with individual antiestrogens and particularly in the presence

of both (82%). In EGF conditions, ICI 164.384 and combination with cyclofenil inhibited proliferative effect of EGF. Similar patterns were noticed in FSH treated cells. Telomerase activity (TA) was higher in SF than in LF-GC. Cyclofenil had no inhibitory effect on TA in SF-GC but decreased TA by 30%-33% in a mixture with ICI 164.384 in LF-GC. In EGF stimulated cells neither individual nor combined treatment had inhibitory effect on TA in SF and LF-GC. In FSH conditions only the combined treatment suppressed TA significantly in SF-GC. Supported by GACR 523/05/2062 and MZE 0002701401.

P20 Isolation of PAG from buffalo (Bubalus bubalis) placenta

Olimpia Barbato[1], Noelita Melo de Sousa[2], Karl Klisch[3], Emeline Clerget[2], Alessandro Debenedetti[1], Vittoria Lucia Barile[4], Alessandro Malfatti[5], Jean François Beckers[2]

[1]*Dip. Scienze Biopatologiche ed Igiene delle produzioni animali e alimentari, Perugia, Italy*

[2]*Physiology of Animal Reproduction, Liege – Belgium*

[3]*Microscopical Anatomy, Medical School, Hannover- Germany*

[4]*Animal Production Research Institute, Monterotondo (RM), Italy*

[5]*Dipartimento di Scienze Veterinarie, Camerino, Italy*

Introduction: Pregnancy-associated glycoproteins (PAG) constitute a large family of glycoproteins expressed by the outer epithelial cell layer of the placenta. In ruminants, they are synthesized by trophoblast binucleate cells and are released in maternal blood during all pregnancy starting from nidation They are considered as reliable markers of pregnancy. PAG are members of the aspartic proteinase family, having high sequence homology with pepsin, chymosin, cathepsin D and E. The aim of the present study was to isolate and characterize PAG molecules extracted from Bubalus bubalis (water buffalo, wb) cotyledons collected at different gestational ages (5 and 8 months).

Methods: Uteri were collected from pregnant buffalo cows immediately after slaughter. Fetal cotyledons were immediately dissected away from caruncular tissue, extensively washed (0.9% NaCl) and stored at -20°C until use. Cotyledons coming from placentas collected at mid-pregnancy (MP-Cot) and late-pregnancy (LP-Cot) were treated separately. The procedure, monitored by RIA, included neutral extraction (phosphate buffer, pH 7.6), acid and ammonium sulphate precipitations, anion exchange (DEAE-Cellulose) and lectin affinity (*Vicia villosa* agglutinin, VVA) chromatographies. Proteins issues from VVA were analyzed by SDS-PAGE and Western blot (anti-PAG from bovine and caprine origins). The most immunoreactive fractions were transferred to a polyvinylidene difluoride (PVDF) membrane for NH2 –microsequence determination.

Results & Discussion: As determined by Western blot, apparent molecular masses of immunoreactive bands from VVA peaks ranged from 59.5 to 75.8 kDa (MP-Cot) and from 57.8 to 80.9 kDa (LP-Cot). Amino-terminal microsequencing of some of the corresponding Coomassie blue stained proteins (62, 68, 70, 73 and 75 kDa) allowed us to identify three distinct wbPAG sequences: RGSXLTIHPLRNIRDFFYVG, RGSXLTILPLRNIID and RGSXLTHLPLRNI. This study demonstrates a multiplicity of PAG expressed in buffalo placenta. The newly characterized wbPAGs and the procedure used to isolate them, will be helpful in producing new antisera for investigating PAG secretion in pregnant buffaloes.

P21 Circulatory cytokine and prolactin profiles throughout murine lactation

Hema Bulsara[1], Katherine Martin[1], A Parlow[2], James Walker[3], Nicolas Orsi[1]

[1]*University of Leeds Perinatal Research Group, Level 4, Leeds Institute of Molecular Medicine, JIF Building, St James's University Hospital, Beckett Street, Leeds, LS9 7TF, UK*

[2]*National Hormone & Peptide Program (NHPP), Harbour-UCLA Medical Center, 1124 W. Carson Street, Torrance, California 90509, USA*

[3]*Department of Obstetrics, Level 9, Gledhow Wing, St James's University Hospital, Beckett Street, Leeds, LS9 7TF, UK*

Introduction: While mouse pregnancy gestation features marked increases in serum cytokine profiles starting mid-pregnancy and peaking at the time of littering, post-partum and lactational profiles are undetermined. This study examined the time frame of cytokine decrease to pre-gestational levels during lactation, and whether this was influenced by the immunomodulatory properties of circulatory prolactin.

Methods: Serum (from blood obtained by cardiac puncture) was collected from naturally-cycling CD1 females and dams throughout lactation (litters standardised to 8 pups): days 1 (<24h littering), 2 (+/- pups), 4 (+/- pups), 10, 16, 21 and 24 (n = >7 mice per group). Individual samples were analysed for prolactin (by radioimmunoassay) and 23 cytokines: interleukins (IL)-1α/β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-10, IL-12 (p40/p70), IL-13, IL-17, eotaxin, granulocyte/granulocyte macrophage-colony stimulating factors, interferon-g, keratinocyte-derived chemokine, monocyte chemoattractant protein-1, macrophage inflammatory proteins-1α/β, regulated upon activation normal T-cell expressed and secreted (RANTES), and tumour necrosis factor-α (by multiplex immunoassay). Data were analysed by one-way ANOVA/Kruskal-Wallis tests, with Fisher's LSD/Mann-Whitney-*U* tests *post hoc*.

Results & Discussion: Prolactin levels were significantly higher than normal cycling levels on days 2 (P<0.05), 4 (peak levels; P<0.01), and 10 (P<0.01) of lactation, returning to pre-gestational levels by day 21. Low levels also characterised animals whose pups had been removed. Cytokine levels were relatively low on day 1 of lactation, indicating a very rapid fall from high peri-partum levels, coinciding with post-partum oestrus. All concentrations increased markedly by days 2 and 4 (except in females without pups), peaking on day 10, and decreasing thereafter (though not to pre-gestational levels). The simultaneous rise in circulatory prolactin and cytokine levels (which incorporates a lag phase) suggests that they may either be (i) subject to common regulatory mechanisms and/or (ii) causally related (with prolactin regulating cytokine profiles).

P22 Effects of Synthetic Prenatal Glucocorticoid Application on foetal and adult programming of the female marmoset monkey

Almuth Einspanier, Christina Schlumbohm, Nicola Beindorff

University Of Leipzig, An Den Tierkliniken 1, Veterinary Faculty, Germany

Introduction: Many disorders occurring in adult life span, like diabetes or hypertension, date back to influences of the intra-uterine environment during prenatal time. Therefore, the aim of the study was to investigate the role of prenatal exposure to glucocorticoids (dexamethasone = DEX) in a non-human primate model, the marmoset monkey.

Methods: 46 pregnancies have been examined by physiological data, endocrine parameters, and ultrasound/color doppler under the influence of daily DEX (5 mg/kg), given orally either during early (day 42-48, n=15) or late in gestation (day 9097, n=13), in comparison with control animals (n=18). Afterwards the post-parturition period of the mother and new born has been followed as well as further development of the female off-spring till sexual maturity.

Results & Discussion: There were no significant differences between the control group and the groups treated with DEX in terms of uterine blood flow as well as growth rate, gestation length, biparietal diameter and body weight. Treatment with DEX late in gestation did not show any significant influence on all analysed hormones compared to the control group. However, due to DEX treatment early in gestation, a significant decline in progesterone and a significant rise in oestradiol during treatment, but no significant influence on relaxin could be detected. All DEX-exposed female offspring showed a significant increase of body weight (456g±86 g, n=10) versus control (344±51 g, n=15) at 20 months of age, likewise an earlier cycle activity (in average 16 months of age) compared to the control group (in average 20 months of age). In summary, significant endocrine effects of early DEX were present during pregnancy. However, there must be minor impact throughout all DEX-treated pregnancies, because effects on the body weight and fertility of the F1-generation were apparent under both DEX-treatments, which suggest an epigenetic effect.

Supported by EU (EUPEAH-Project).

P23 AI outcome and metabolic indicators in cows: a field study

Gianfranco Gabai[1], Olimpia Barbato[2], Licia Ravarotto[3], Sara Centenaro[4], Mariacristina Merlo[1], Antonio Mollo[4]

[1]*Dipartimento di Scienze Sperimentali Veterinarie, Univ. Padova, Italy*

[2]*Dipartimento di Scienze Biopatologiche ed Igiene delle Produzioni Animali ed Alimentari, Univ. Perugia,Italy*

[3]*Istituto Zooprofilattico Sperimentale delle Venezie, Padova,Italy*

[4]*Dipartimento di Scienze Cliniche Veterinarie, Univ. Padova,Italy*

Introduction: The AI outcome can be affected by nutritional and metabolic conditions that may disturb either CL function and embryo implantation leading to return to estrus. In this work, we investigated the relationship between AI outcome, blood metabolites and plasma progesterone from AI to pregnancy diagnosis.

Methods: The AI outcome (N=143) performed in 89 cows belonging to 3 herds was recorded for 6 months. The diagnosis of pregnancy was performed by ultrasonography at day 28-34. The outcome of 81 AI was monitored also by PAG measurement in mother blood (RIA) after day 25 to assess embryo viability. Plasma P4, urea, NH3, cholesterol, triglycerides, NEFA, βOH-butyrate, and glucose were measured every week from AI to the pregnancy diagnosis. The relationship between AI outcome and metabolic indicators was analysed by ANOVA.

Results & Discussion: 48.3% of cows became pregnant within 3 AI, 21.3% did not become pregnant after 3 or more AI, and only 3.4% became pregnant beyond 3 AI. 27% of animals did not become pregnant within the end of the experiment. In 14 cases, plasma PAG concentrations measured before a negative ultrasonographic diagnosis of pregnancy were compatible with the presence of a viable embryo thus suggesting a possible embrionic loss. In other 6 cases, plasma PAG were actually decreasing from day 25 onward, indicating that embryo death occurred. Plasma progesterone tended to be lower in negative AI only on day 18. Plasma triglicerydes concentration and NH_3/urea molar ratio were slightly but significantly ($P<0.05$) lower in positive AI. It is unlikely that these differences can affect the establishment of pregnancy "per se", but they may be the signal of more subtile mechanisms. Measurement of plasma PAG in association with the ultrasonographic diagnosis of pregnancy may be an important tool to identify embryonic death.

Supported by Regione Veneto, I.

P24 Relationship between return to oestrus, luteal function and metabolic indicators in dairy cows

Mariacristina Merlo[1], Antonio Mollo[2], Angela Tramontano[2], Sara Faggin[1], Paolo Dal Vit[3], Igino Andrighetto[3], Gianfranco Gabai[1]

[1]*Dipartimento di Scienze Sperimentali Veterinarie – Padova, I, Italy*

[2]*Dipartimento di Scienze Cliniche Veterinarie – Padova, I, Italy*

[3]*Istituto Zooprofilattico Sperimentale delle Venezie – Padova, I, Italy*

Introduction: Many factors may disturb oocyte fertilization and embryonic development giving rise to return to oestrus. Among these factors, CL function at the time of embryo implantation seems to have a major role, and luteal activity can be affected by nutritional and metabolic conditions. In this study, we investigated the relationship between AI outcome, CL activity and plasma metabolic indicators from AI to the time of expected embryo implantation.

Methods: Whey progesterone measured every 3-4 days by RIA was used to study the ovarian cycle characteristics in 33 dairy cows from day 15 post-partum to pregnancy diagnosis performed at day 30-35 after each AI. Animals were monitored until they became pregnant or for at least three consecutive AI. The whole observation period lasted seven months. Plasma progesterone, urea, cholesterol, triglycerides, NEFA, βOH-butyrate and glucose were measured in samples taken at AI (day 0) and 7, 14 and 21 days after AI.

Results & Discussion: 66.7% of cows became pregnant within 3 AI, 18.2% did not become pregnant within 3 AI and 15.1% did not become pregnant within the observation period. Mean plasma NEFA at the time of the first AI were at basal levels suggesting that cows were inseminated once overcome

the period of negative energy balance. Plasma progesterone was significantly lower on day 14 in AI with negative outcome (5,11+/-0,68 vs 7,42+/-0,69 ng/ml; *P*<0,05). Negative AI were classified according to ovarian cycle length subsequent the AI (Physiological, N=11, 22,0+/-0,6 days; Long, N=14, 34,8+/-3,0 days). Plasma progesterone was significantly lower (*P*<0,05) after negative AI followed by a Physiological cycle only on day 21. Triglycerides and cholesterol were slightly but significantly higher in negative AI (*P*<0,01). These differences may imply the presence of metabolic signals affecting CL activity and/or embryo survival.

Supported by Regione Veneto, I.

P25 The role of transcription factor p53 in regulation by ghrelin of apoptosis, proliferation, and secretion in ovary

Andrej Benèo[1,2], Alexander Sirotkin[1,2], Silvia Pavlová[1,2], Ján Kotwica[3]

[1]*Dept. Genetics & Reproduction, Slovak Centre of Agricultural Studies, Nitra, Slovakia*

[2]*Dept. Zoology & Anthropology, Constantine the Philosopher University, Nitra, Slovakia*

[3]*Institute of Animal Production and Food Research, Olsztyn-Kortovo, Poland*

Introduction: The aim of this study was to examine the role of transcription factor p53 on proliferation (accumulation of PCNA, a marker of proliferation), apoptosis (expression of apoptosis-stimulated kinase, ASK-1) and the release of progesterone (P_4), prostaglandin F (PGF) and oxytocin (OT) by porcine ovarian granulosa cells cultured with or without the hormone ghrelin (G). These parameters were analysed by using immunocytochemistry, Western blotting and radioimmunoassay.

Methods: Transfection of granulosa cells by a gene construct encoding p53 promoted the accumulation of p53 and decreased the accumulation of PCNA and ASK-1. It also increased progesterone secretion but decreased PGF and OT secretion.

Addition of ghrelin (0, 1, 10 or 100 ng/ml) increased the accumulation PCNA, ASK-1 and p53 and the secretion of OT, P_4 and PGF. Over-expression of p53 reversed the effect of G on PCNA, ASK-1 and p53 and OT from stimulation to inhibition but did not modify the effect of G on PGF or P_4.

Results & Discussion: These results suggest (1) that transcription factor p53 may be involved in regulating porcine ovarian function, activating both proliferation and apoptosis and promote or suppressing the secretion of P_4, PGF and OT, (2) that G can control ovarian functions, activating proliferation, apoptosis, and hormones release, and (3) that p53 can mediate G action.

P26 The involvement of transcription factor STAT – 1 in the regulation of porcine ovarian cell functions

Silvia Pavlová, Alexander Sirotkin, Andrej Benco, Dusan Vasicek

University Of Constantine Philosopher, Nabrezie Mladeze 91, Nitra 949 01, Dobsinskeho 7, Lutila 96622, Slovakia

Introduction: The aim of this study was to examine the involvement of transcription factor STAT-1 in the control of ovarian function. The activity of porcine ovarian granulosa cells, transfected or non-transfected with a cDNA construct encoding STAT-1, cultured with and without leptin and ghrelin was evaluated.

Methods: Porcine ovarian granulosa cells were obtained from non-cycling pigs, transfected with a plasmid inducing over-expression of STAT-1 and cultured until a monolayer was formed. Thereafter the cells were cultured in presence or absence of either leptin or ghrelin (0, 1, 10, or 100 ng/ml medium). The expression of STAT-1, apoptosis-associated substances (the pro-apoptotic peptide bax and the apoptosis-stimulated kinase ASK-1), a marker of proliferation (the proliferating cell nuclear antigen, PCNA), secretion of progesterone (P_4) and accumulation of CREB-1 were analyzed using immunocytochemistry, Western immunoblotting and RIA

Results & Discussion: It was observed that the transfection of cultured porcine ovarian granulosa cells with the gene construct encoding STAT-1 stimulated accumulation of STAT-1, increased the occurrence of both apoptosis and proliferation and inhibited the secretion of P_4 and accumulation of CREB-1. Addition of leptin or ghrelin was able to affect proliferation, apoptosis and secretion of P_4, whilst transfection of cells with the STAT-1 gene construct substantially modified the response of cells to exogenous leptin and ghrelin. These results suggest the involvement of transcription factor STAT-1 and the hormones ghrelin and leptin in the control of proliferation, apoptosis, secretory activity and expression of transcription factor CREB-1 by ovarian cells. They also suggest that STAT-1 mediates the actions of leptin and ghrelin.

P27 Inverdale gene affects progesterone levels in ewe lambs

Frances Alink[1,3], Linda Mitchell[1], John Robinson[1], Robert Watt[1], Morag Ewen[1], James Mylne[2], Maureen Wood[3], Tom McEvoy[1]

[1]*Scottish Agricultural College, Sustainable Livestock Systems Group, Craibstone Estate, Aberdeen, AB21 9YA, UK*

[2]*Britbreed Ltd, Ormiston, East Lothian, EH35 5NG, UK*

[3]*University of Aberdeen, Assisted Reproduction Unit, Foresterhill, Aberdeen, AB25 2ZD, UK*

Introduction: The Inverdale fecundity gene is located on the X-chromosome. Heterozygous carriers of the gene show an increase of approximately 1.0 in their number of ovulations. The present study tested for effects of maternal nutrition during fetal life on luteal function in Inverdale gene carrier and non-carrier ewe lambs.

Methods: Spring-born Texel x Scottish Blackface ewe lambs (n=80) that were either carriers (C, n=37) or non-carriers (NC, n=43) of the Inverdale gene and whose dams had been given a diet supplying either 0.5 maintenance (0.5 M) or 1.0 maintenance (1.0 M) during the first 95 days of gestation were managed as a single flock. They were bloodsampled twice weekly in the 7 week period preceding the winter solstice. Plasma was assayed for progesterone and levels equal to or higher than 1.0 ng/ml indicated the presence of a corpus luteum. Data were analysed using ANOVA (Genstat; Version 8).

Results & Discussion: By the end of the sampling period 92 and 85% of the NC ewe lambs from the 0.5 M and 1.0 M treatments, respectively had luteal activity. Corresponding values for the C ewe lambs were 100 and 78%. The mean progesterone values (+/-SEM) over the sampling period were 4.0+/-0.24 and 4.4+/-0.34 for the NC ewe lambs from the 0.5 M and 1.0 M treatments, respectively. The corresponding values for the C ewe lambs were 3.0+/-0.33 and 3.4+/-0.28, respectively (Genotype: P<0.005; Maternal nutrition: ns; Interaction: ns). Despite their presumptive higher ovulation rate ewe lambs carrying the Inverdale gene had lower circulating progesterone concentrations than their non-carrier counterparts. This finding could have implications for nutritional management during the peri-conception period as high plane feeding suppresses progesterone concentrations in the ewe.

Funded by SEERAD; FMA is a Genesis Faraday Associate and supported by Innovis and The SAC Trust.

P28 LH peak in oestrus synchronized buffaloes

Vittoria Lucia Barile[1], Corrado Pacelli[2], Giuseppina Maria Terzano[1], Antonio Borghese[1], Olimpia Barbato[3], Luca Todini[3], Alessandro Malfatti[4]

[1]*Istituto Sperimentale per la Zootecnia, Monterotondo – Roma, Italy*

[2]*Dipartimento di Scienze delle Produzioni Animali, Università della Basilicata, Potenza, Italy*

[3]*Dipartimento di Scienze Biopatologiche Veterinarie, Università di Perugia, Italy*

[4]*Dipartimento di Scienze Veterinarie, Università di Camerino, Italy*

Introduction: Artificial insemination (AI) is necessary to promote genetic improvement in farm animals, but problems of oestrus detection limit its application in buffalo herds. Furthermore, because buffalo is a species with seasonal anoestrus, farmers are inclined to natural mating in order to avoid the risk to have non-pregnant buffaloes and lose the production of a whole year. In order to increase the use of AI without utilizing oestrus identification tecniques, different hormonal treatment schedules and fixed time AI have been proposed.

Methods: The aim of the work was to evaluate LH peak and ovulation time in buffalo cows submitted to two different hormonal protocols for fixed time insemination in different breeding period. Twenty-four lactating buffaloes, were equally assigned to two different oestrus synchronization treatments, PRID plus PMSG and $PGF_{2\alpha}$ or Ovsynch, in two different period of the year: February (end of the natural breeding period) and May (beginning of the non-breeding period). Blood samples were taken at 4 hours intervals, starting 24 hours after PRID removal or 12 hours from $PGF_{2\alpha}$ injection in the Ovsynch (considered as the end of the treatments) until the 108th hour. Plasma LH concentrations were determined by ELISA (LH DETECT, INRA, France). Ovulation was assessed by ovarian ultrasound monitoring. Data were analyzed by ANOVA.

Results & Discussion: No differences were found between treatments or period. LH peak occurred at 47.60±1.71 h from PRID removal and at 50.80±1.87 h from $PGF_{2\alpha}$ in the Ovsynch protocol, and at 50.90±1.79 and 47.50±1.79 from the end of treatments in February and May respectively. The interval between LH peak and ovulation was: 29.33±8.06 h in the PRID protocol and 30.22±6.03 h in the Ovsynch one, 30.00±4.32 h in February and 28.40±8.93 h in May. Both treatments were able to induce LH peak and synchronize oestrus also during the non-breeding period.

P29 SNPs in the bovine luteinising hormone receptor

N Hastings[2], S Donn[2], K Derecka[1], JA Woolliams[2], APF Flint[1]

[1]*School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, LE12 5RD, UK*

[2]*Roslin Institute, Roslin, Midlothian, Edinburgh, EH25 9PS, UK*

Introduction: There is an unfavourable genetic correlation between milk yield and fertility in dairy cattle, and recent selection for yield has resulted in reduced fertility. Conception to first service rates have declined during the past 20 years at 1% per year, and trends in estimated breeding values (EBVs) for calving interval (CI), days in milk to first service (DFS), non-return rate 56 days after first service (NR56) and number of inseminations required per conception (CINS) all reflect decreased fertility. These trends have stimulated the recent development of a fertility index for UK bulls, providing EBVs for daughter fertility. We have examined a number of candidate genes involved in reproduction for polymorphisms associated with fertility. Here we report single nucleotide polymorphisms (SNPs) in the luteinising hormone receptor (LHR).

Methods: DNA prepared from semen samples of a sub-set of bulls represented in the fertility index was amplified by polymerase chain reaction and sequenced. Associations between SNPs and EBVs for fertility traits were tested by multivariate analysis.

Results & Discussion: Three SNPs were detected in exon 11 of the LHR, a mis-sense mutation (TGG-TGT) at codon 467, a silent mutation (CTC-CTT) at codon 490 and a mis-sense mutation (CAG-CAT) at codon 527. These resulted in substitutions of cysteine for tryptophan (W467C) and histidine for glutamine (Q527H). A minimum of 4 haplotypes, GCG, GTG, TCG and TCT at positions 467, 490 and 527, were sufficient to explain the data. These occurred at frequencies of 0.75, 0.25, 0.0 and 0.0 in British Friesian bulls and 0.44, 0.32, 0.15 and 0.09 in Holsteins. Haplotype substitution was associated with changes in fertility. In particular the TCT haplotype decreased CI and DIM1 EBVs by 2.58 – 5.00 and 1.59 – 3.04 days respectively, with no effects on NR56 or CINS. Genotyping at the LHR could therefore be used to improve dairy cow fertility.

P30 Evaluation of an existing model for the prediction of response to clomifene citrate

Cyrus Ghobadi[1,2], Tien Nguyen[1,2], Saad Amer[2], Amin Rostami-Hodjegan[1], Martin S. Lennard[1], William L. Ledger[2]

[1]*Academic Unit of Clinical Pharmacology, University of Sheffield, UK*

[2]*Academic Unit of Reproductive And Developmental Medicine, University of Sheffield, UK*

Introduction: Clomifene is the first line medication for normogonadotropic oligo/amenorrheic infertility (WHO II). A nomgram was developed to predict clomifene response in this group based on initial characteristics such as Free Androgen Index (FAI), Body Mass Index (BMI) and cycle history. However, no validation was done in an independent set of patients. This study was undertaken to evaluate accuracy of the nomogram to identify clomifene resistant patients a priori.

Methods: 315 individual notes were retrospectively reviewed. Age, BMI, FAI, cycle history and chances of ovulation predicted by nomogram were documented. The relationship between predicted outcomes and the occurrence of ovulation, confirmed by day 21 serum progesterone levels, were examined computing diagnostic test characteristics and Kappa Statistics.

Results & Discussion: Of the 315 cases, 156 did not fulfil the criteria and excluded, 55 had inadequate data and 104 cases were included. Sensitivity, the probability that the nomogram identifies a clomifene responder among patients who clinically ovulated=92%(95%CI:85-99); Specificity, the probability that the nomogram identifies a clomifene resistant among patients who did not ovulate=31%(95%CI:16-47); Positive Predictive Value, the probability that a patient ovulates if identified as a clomifene responder by the nomogram=73%(95%CI:65-89); Negative Predictive Value, the probability that a patient will not ovulate if identified as a clomifene resistant by the nomogram=80%(95%CI: 60-99); Kappa measure of agreement between the nomogram and progesterone concentration= 0.26(95%CI:9-44). The nomogram was not suitable for patients with screening values outside the range. The probability that nomogram could identify clomifene resistants was high. However, the 95%CI was not narrow enough to predict this possibility accurately. Nomogram could not identify appropriate dose to achieve ovulation. Although the nomogram appeared not to be sufficiently predictive in Jessop Hospital Fertility Clinic in Sheffield but it showed better performance in clinical settings with relatively lower CC response rates.

P31 Relations between milk yield and concentration of IGF-I in the blood plasma of ewes in dependence of the reproduction status

Jutta Gottschalk, Thomas Hippel, Almuth Einspanier, Eberhard Grün, Shehadeh Kaskous

Institute Of Physiological Chemistry, Veterinary Faculty, University Of Leipzig, An Den Tierkliniken 1, Leipzig, Germany

Introduction: In the present study the relationship between milk yield and concentration of IGF-I in the blood plasma as well as number of suckling lambs were examined in 11 Awassi ewes (14-16 months of age, in average 41 kg body weight) in Syria to obtain basic physiological knowledge on the endocrinological control of lactation for this sheep race.

Methods: Plasma IGF-I of 8 lactating ewes (3 with twins, 5 with a single lamb) and 3 non-lactating animals (controls) was measured weekly and compared with the milk yield during the whole lactating period (7th to 161st day of lactation) as well as evaluated separately for suckling (7th to 63th day) and hand milking period (70th to 161th day).

Results & Discussion: IGF-I concentration of lactating ewes at birth (328 +/- 39 ng/ml) was significantly higher than for control animals (208 +/- 28 ng/ml). No differences were observed during the suckling period, whereas IGF-I levels of lactating ewes (186 +/- 13 ng/ml) were significantly lower than of the control group (205 +/- 22 ng/ml) throughout milking period. Ewes with twins had higher concentrations of IGF-I (231 +/- 19 ng/ml) than non-lactating animals (202 +/- 21 ng/ml) in the suckling period as well as in the lactation period and also higher levels of IGF-I in all phases of lactation than animals suckling single lambs. In accordance to milk yield, ewes with twins had significant higher milk production than ewes with singleton during the suckling period, but in general both had the same milk production throughout the whole milking period. In summary, IGF-I concentrations of the blood plasma showed a strong correlation with the milk yield ($r=0.71$, $p<0.001$ and $r=0.89$, $p<0.001$, respectively) during the whole lactating period and in particularly during the milking period.

P32 Comparative study on the proliferative activity of the OSE

Salina Saddick[1], Evelyn Telfer[1], Hamish Fraser[2]

[1]*Institute of Cell and Molecular Biology, University of Edinburgh, The Darwin Building Mayfield Road, Edinburgh EH9 3JR, UK*

[2]*MRC Human Reproductive Sciences Unit, 47 Little France Crescent, Edinburgh UK*

Introduction: The ovarian surface epithelium (OSE) is a single layer of cells covering the entire surface of the ovary. Although the OSE comprises only a small fraction of the ovary, they have been strongly implicated in the genesis of common ovarian cancers. The aim of this study was to determine the proliferative activity of the OSE throughout the ovulatory cycle, during pregnancy and after GnRH antagonist treatment in adult marmoset monkeys.

Methods: Cell proliferation was monitored using detection of DNA-incorporation of bromodeoxyuridine (BrdU) by immununohistochemistry. In addition cell death was detected by immunolocalization of the apoptosis-related gene Caspase-3. Ovaries were collected at the mid- and late follicular phase, mid-luteal phase and days 21 and 28 of pregnancy (n=8 per group). GnRH antagonist was administrated on day 0 of the follicular phase and ovaries studied on day 10 (n=6). Ovaries from control marmosets were studied at day 5 (mid follicular phase). All animals were injected with BrdU 1h prior to collection of the ovaries.

Results & Discussion: Cumulative BrdU-labeling demonstrated that OSE cells proliferate at a low level with only 0.48 % of cells taking up BrdU during the follicular phase (control). Proliferation was further reduced during pregnancy and after GnRH antagonist treatment where OSE cells showed negligible BrdU labelling (0.007%, 0.02%) respectively ($p \leq 0.05$). Caspase-3 was not expressed in any of the OSE cells during the follicular and luteal phase it was expressed only in granulosa cells of atretic follicles. The results show that proliferative activity of the OSE is down regulated during pregnancy and after GnRH antagonist-induced suppression of ovarian activity. This supports the hypothesis that OSE proliferation in the marmoset is regulated primarily via endocrine mechanisms.

P33 Mammalian lignan content of milk from cattle fed a linseed-enriched diet

Maurice Sauer[3], Ai Qin Zhang[1], Laurence Howells[2]

VLA-Weybridge, Woodham Lane, New Haw, Addlestone, KT15 3NB, UK

Introduction: Linseed represents a rich source of n-3 polyunsaturated fatty acids (PUFAs). The consumption of diets high in PUFAs is associated with decreased incidence of cardiovascular disorders. One means of aiding such a shift in consumer diet would be to increase PUFAs in e.g. milk by feeding livestock rations rich in PUFAs. (Abayasekara & Wathes, 1999). Linseed also represents a rich source of the plant lignans which are metabolised by gut micro-flora to the 'mammalian' lignans enterodiol (ED) and enterolactone (EL) which are found in high concentrations in urine and milk. Lignans bind to SHBG and may have weak oestrogenic or anti-oestrogenic activity *in vivo* and *in vitro*.

Methods: We evaluated whether feeding cattle a diet rich in linseed, to modify milk PUFAs, results in a concomitant increase in mammalian lignan content. Such changes may have implications for consumer exposure to these putative phytoestrogens.

Four first lactation heifers were fed standard rations, but for a continuous three month period on the high linseed diet. Milk samples were collected throughout and analysed for mammalian lignan content by LC-MS/MS.

Results & Discussion: EL and ED were detected in milk, largely in conjugated (EL 96%) form. EL concentrations were ≥ 10x those of ED. While on the standard diet, EL concentrations in three animals (26.3 +/- 4.8 ng/ml), were consistent with that in retail whole milk (41+/- 4 ng/ml), but in the forth, content was substantially higher (122 ng/ml). The high linseed diet increased EL 4-6 fold within 2 days, but interestingly content reduced thereafter, over 1 month, to ~ control concentrations. Reversion to the standard diet after 3 months, gave an initial decrease of EL, then a small but consistent rise. Potency considerations suggest that at these concentrations, direct oestrogenic effects on the consumer are unlikely.

P34 Effect of hemi-ovariectomy on FSH, oestradiol and inhibin

Anongnart Somchit[1,3], Bruce K Campbell[4], Muhammad Khalid[2], Rex J Scaramuzzi[1]

[1]*Department of Veterinary Basic Sciences, Royal Veterinary College, London, UK*

[2]*Department of Veterinary Clinical Sciences, Royal Veterinary College, London, UK*

[3]*Department of Animal Husbandry, Chulalongkorn University, Bangkok, Thailand*

[4]*Division of Obstetrics and Gynaecology, University of Nottingham, Nottingham, UK*

Introduction: Hormonal feedback from the ovary to the hypothalamo-pituitary axis is provided by oestradiol, progesterone and inhibin and they negatively regulate the secretion of follicle-stimulating hormone (FSH) and luteinising hormone. Removal of one ovary leads to an immediate decrease in negative feedback and the hypothalamo-pituitary system responds by increasing its secretion of FSH, leading to the restoration of normal folliculogenesis. This study investigated the effects of hemi-ovariectomy in cyclic ewes on plasma FSH and oestradiol-17β and follicular fluid oestradiol-17β and inhibin A.

Methods: Sixteen, cyclic Welsh Mountain ewes were randomly divided into 2 groups: hemi-ovariectomised and sham-operated. Hemi-ovariectomy was performed on day 13 of the oestrous cycle in 8 ewes and the other 8 ewes were sham-operated. Blood was collected from the jugular vein every hour from 6h before until 30h after surgery; plasma was separated and stored at -20°C. Animals were euthanised 30h after surgery their ovaries immediately collected and all follicles dissected and classified as small (>1.0–3.5mm diameter) or large (>3.5 mm). Follicular fluid was collected and stored at -20°C. Plasma was assayed for FSH and oestradiol-17β and follicular fluid for oestradiol-17β and inhibin A.

Results & Discussion: FSH was higher in hemi-ovariectomised ewes (P=0.036). Plasma oestradiol-17β concentrations tended to be higher in shams (P=0.121). Follicular fluid oestradiol-17β in follicles <3.5 mm in hemi-ovariectomised ewes (273+/-156 ng/ml; mean +/- SEM) was higher (P=0.035) than in shams (52.1+/-13.4 ng/ml) but not in follicles ≥3.5 mm (hemi-ovariectomy 1613+/-466 and shams 994+/-325 ng/ml; P=0.292). Inhibin A in follicles ≥3.5 mm from hemi-ovariectomised and sham-operated groups was 57.1+/-17.3 and 53.7+/-18.6 μg/ml, respectively. In conclusion, hemi-ovariectomy of cyclic ewes increases FSH and it results in increased oestradiol-17β in small (<3.5mm) but not large (≥3.5mm) follicles.

A Somchit is supported by a scholarship from the Royal Thai Government.

P35 Expression of PG-9-KR in pig endometrium and trophoblast

Agnieszka Waclawik, J Klos, AJ Ziecik

Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, 10-747 Olsztyn, Poland

Introduction: The prostaglandin E2/prostaglandin $F_{2\alpha}$ ($PGE_2/PGF_{2\alpha}$) ratio plays an important role in regulation of the oestrous cycle and establishment of pregnancy. In many species, including the pig, both conceptus and endometrium synthesise PGE_2, which may antagonise luteolytic $PGF_{2\alpha}$ Recently, we have reported the expression profiles of terminal prostaglandin synthases in porcine endometrium and trophoblast (Waclawik et al., 2006. Endocrinology 147:210-221). In present study the expression patterns of prostaglandin 9-ketoreductase (PG-9-KR), the enzyme converting PGE_2 into $PGF_{2\alpha}$,were investigated in endometrium and conceptus/trophoblast in the pig.

Methods: Endometrial samples were analyzed from gilts on days 1-21 of the oestrous cycle (n=37) and on days 10-25 of pregnancy (n=34). Conceptuses were collected on days 10-19 and trophoblasts on days 20-25 of pregnancy. PG-9-KR mRNA expression was examined by quantitative RT-PCR, whereas the protein levels were analysed by Western blotting.

Results & Discussion: Quantification of PG-9-KR mRNA revealed no significant variation in endometrium throughout the oestrous cycle; however, an increase in protein expression on days 16-17 (mean+/-SEM, 3.6+/-0.9; $p<0.05$), when compared with days 1-8 (0.8+/-0.1) and days 18-21 (1.4+/-0.3), was observed. In pregnancy, profiles of endometrial PG-9-KR mRNA and protein were parallel with maximum on days 24-25. Endometrial PG-9-KR protein levels on days 10-11 and 16-17 of pregnancy were significantly

lower when compared with corresponding stages of the oestrous cycle. In day 10-13 conceptuses, PG-9-KR mRNA and protein levels were very low (0.03+/-0.007) and undetected, respectively. Afterwards, high expression of PG-9-KR mRNA (20-fold increase) and protein was observed in conceptuses/trophoblasts on days 14-25. Down-regulation of PG-9-KR in endometrium and conceptus on days 10-11 of pregnancy may be involved in increasing the PGE_2/PGF_2 ratio necessary for the maternal recognition and establishment of pregnancy. Summarising, this is a novel report characterising the functional changes of expression of endometrial and conceptus/trophoblast PG-9-KR in the pig.

P36 Booroola mutation alters FSH release from pituitary cells

Julia Young[1,2], Jenny Juengel[3], Ken Dodds[4], Peter Dearden[1],Theresa Wilson[2], Kenneth McNatty[3]

[1]*Department of Biochemistry, University of Otago, Dunedin, New Zealand*

[2]*AgResearch Molecular Biology Unit, Department of Biochemistry, University of Otago, Dunedin, New Zealand*

[3]*AgResearch, Wallaceville Animal Research Centre, Upper Hutt, New Zealand*

[4]*AgResearch, Invermay Agricultural Centre, Mosgiel, New Zealand*

Introduction: Bone morphogenetic proteins (BMP) are thought to be involved in regulating FSH synthesis and secretion at the level of the pituitary. Pituitary cells harvested from homozygous (BB) Booroola and wild-type (++) ewes were examined in vitro to determine if the mutation in the BMPRIB receptor in Booroola sheep alters FSH secretion.

Methods: Cells were collected from ewes (n=37) 24 hrs following induction of luteolysis (n = 5-6 pools per genotype) and cultured for 72 hrs before being challenged for 24 hrs with BMP2, BMP4, BMP6, growth and differentiation factor-9 (GDF9), transforming growth factor-β 1 (TGFβ1), and GnRH. The levels of FSH were measured by RIA and compared to the untreated controls.

Results & Discussion: GnRH (10-1000 $ng.ml^{-1}$) increased FSH secretion in both genotypes ($P<0.001$). The lowest dose of BMP2 (10 $ng.ml^{-1}$) suppressed FSH secretion by 30-35% in both ++ ($P<0.01$) and BB ($P<0.001$) cells. Continued suppression across increasing doses were only observed in BB cells ($P<0.001$). The response to BMP2 at 100 and 1000 $ng.ml^{-1}$ differed between BB and ++ cells ($P<0.05$). Response to BMP4 differed between genotypes ($P<0.01$) with significant suppression ($P<0.001$; up to 36%) observed in BB but not ++ ($P>0.05$; 15%) cells. BMP6 suppressed FSH concentrations (up to 35%) at 10 ($P<0.01$) and 1000 ($P<0.001$) $ng.ml^{-1}$ in BB cells and at 1000 $ng.ml^{-1}$ in ++ cells ($P<0.01$; 25%) with a genotype difference only at the lowest dose ($P<0.05$). There was also no genotype effect for GDF9, although overall a slight suppression (20%) was noted, reaching significance in the BB cells ($P<0.05$ to $P<0.01$). TGFβ1 did not affect FSH secretion.

In conclusion, BMPs tended to suppress FSH secretion and this effect was enhanced in BB cells.

P37 Effect of Thymoquinone on malformations and oxidative stress-induced diabetic mice

Maher Al-Enazi

Department of Medical Laboratory Sciences, College of Applied Medical Sciences, Aljouf University, Sakaka, Aljouf, Saudi Arabia

Introduction: Pregnancy during diabetes is reported to be associated with several embryological defects. The pathogenesis includes fetal malformations, absorptions and abortions due to increased excessive production of reactive oxygen species. Literature reports have suggested antioxidants to inhibit the genesis of free radicals and decrease the rate of malformations, absorptions and abortions.

Methods: The present study was undertaken to investigate the effect of Thymoquinone (THQ) (a proven antioxidant and an active ingredient of Nigella sativa L.) on embryonic development in streptozotocin (STZ)-induced diabetic mice. Virgin female mice were injected with 45 mg/kg STZ before mating and divided into two groups, as follows (1) The mice treated with STZ alone and (2) mice treated with STZ and THQ (10 mg/kg/day). A parallel control group of animals were simultaneously maintained. All mice were killed on day 19 of pregnancy. The fetuses were analyzed for their (i) weight and malformations and (ii) malondialdehyde (MDA) and glutathione (GSH) concentrations were estimated in the maternal liver.

Results & Discussion: In mice treated with STZ alone the percentages of re-absorptions and malformations were 26.4 and 27.9% respectively, as compared to 1.8 and 2.5% observed in the control group. In group of mice treated with STZ and THQ, the percent reabsorptions and malformations were 9.8 and 12.3 respectively. These results were confirmed by our study on the estimation of MDA and GSH which were significantly increased ($P<0.01$) and decreased ($P<0.001$) respectively. Our data demonstrate that the treatment of thymoquinone during pregnancy of diabetic mice inhibit the rate of embryo malformations by reducing the free radicals, in addition to increasing the size and maturation of embryos. Thus use of antioxidant compounds such as THQ is useful in pregnancy of diabetic females.

Acknowledgement
The author is grateful to the Research Center, College of Pharmacy, King Saud University for financial support (C.P.R.C. 141).

P38 Effects of IVC on bovine cotyledon gene expression

John Rooke[1], Stephen Carmichael[2], Richard Talbot[2], Tom McEvoy[1]

[1]*Sustainable Livestock Systems, SAC, West Mains Road, Edinburgh EH9 3JG, UK*

[2]*ARK-Genomics, Roslin Institute, Roslin, Midlothian EH25 9PS, UK*

Introduction: In previous studies we have found that incubation of zygotes in lipid-containing media reduced blastocyst yield and after transfer of single embryos to recipients influenced fetal development. In vitro culture (IVC) of embryos has also been associated with abnormal placentation. In this experiment the effects of differing IVC conditions on placental gene expression were assessed using microarrays.

Methods: Fetal cotyledons were recovered and snap frozen in liquid nitrogen 63 days after synchronous transfer of day 7 single embryos cultured in either synthetic oviductal fluid plus amino acids and 0.3% albumin (SOF) or SOF to which bovine lipoproteins (2% SOFLP) or the antioxidant, Trolox (0.1mM, SOFT) had been added. Control pregnancies were established by artificial insemination (AI). Microarray analysis was carried out using standard procedures (www.ark-genomics.org). Briefly, RNA was extracted using Trizol and reverse transcribed using the Fairplay II kit to incorporate amino-allyl UTP into cDNA which was labelled with either Cy3 or Cy5. Labelled cDNA was hybridised to bovine 15K microarrays. Each of the three individual IVC treatments was hybridised with control (AI) cDNA including dye reversal (5 replicates / pairwise comparison). Scanned images were analysed using Bluefuse software and statistical analysis was carried out using GeneSpring.

Results & Discussion: When individual IVC treatments were compared by t-tests with AI controls, no significant down-regulation of gene expression was observed and less than 20 genes were significantly ($P<0.05$) up regulated (SOF 19, SOFLP, 15 and SOFT, 14). However when IVC treatments were compared by analysis of variance there were significant ($P<0.05$) differences between treatments with 63 (SOFLP), 33 (SOF) and 16 (SOFT) genes being differentially expressed. These observations suggest that effects of IVC on cotyledon gene expression were variable and treatment dependant.

SAC receives financial assistance from Scottish Executive Environment and Rural Affairs Department.

P39 Ageing and adrenomedullin in the male reproductive system

Fai Tang[1], Yuk Yin Li[1], Wai Sum O[2]

[1]*Department of Physiology, the Unviersity of Hong Kong, Hong Kong*

[2]*Department of Anatomy, the University of Hong Kong, Hong Kong*

Introduction: Adrenomedullin (AM), a vasoactive peptide, has been found in the human and rat male reproductive systems. The present study investigates the age-related changes in the gene expression of AM, its receptor (CRLR, calcitonin receptor-like receptor) and its receptor activity modifying proteins (RAMPs) in the rat testis, the ventral prostate and the seminal vesicle.

Methods: Male S.D. rats aged 3 months (young), 12 months (middle-aged) and 20 months (old) were used. AM levels in the plasma, the testis, the ventral prostate and the seminal vesicle were measured by RIA and the mRNA levels of preproAM, CRLR and RAMPs by RT-PCR.

Results & Discussion: Plasma AM levels were higher in the middle-aged and old rats than in young rats. Testicular AM concentrations as well as the mRNA levels of preproAM and RAMP1 and RAMP3 increased with age. However, the AM concentrations in both the ventral prostate and the seminal vesicle decreased with age. In the ventral prostate, the mRNA levels of preproAM and RAMP1 declined with age while in the seminal vesicle, the mRNA levels of preproAM, RAMPP2, RAMP3 and CRLR were reduced with age. These results demonstrate that aging has different effects on the levels of AM and its receptors in the testis and the accessory sex glands. AM may regulate the functions of these organs and its relative importance may change during ageing.

(This study was supported by a CRCG grant from the University of Hong Kong).

P40 Testicular adrenomedullin and endothelin interaction

Yuen Fan Chan[1], Wai Sum O[1], Fai Tang[2]

[1]*Department of Anatomy, Department Of Physiology, Faculty of Medicine, The University of Hong Kong, 21 Sassoon Road, Pokfulam, Hong Kong*

[2]*Department of Physiology, The University of Hong Kong, 21 Sassoon Road, Pokfulam, Hong Kong*

Introduction: Adrenomedullin (AM), a novel hypotensive peptide, is a peptide hormone that potentially regulates testicular functions. AM is known to antagonize the actions of endothelin-1 (ET-1) in many organs or tissues such as the vasculature. It is hypothesized that AM may exert autocrine/paracrine actions in the testis that are antagonistic to those of ET-1. The effect of AM on Leydig cell steroidogenesis and its interaction with ET-1 in the testis were studied.

Methods: Sertoli and Leydig cells were isolated from Sprague-Dawley rat. The effects of AM on basal, HCG-stimulated and ET-1-stimulated Leydig cell steroidogenesis were studied by measuring testosterone production using enzyme immunoassay. The effects of AM and ET-1 on the production of each other in the isolated cells were also studied. The expression levels of prepoAM and prepoET-1 mRNA in the cells were studied by semi-quantitative PCR and the levels of AM and ET-1 secreted by the cells were studied by radioimmunoassay.

Results & Discussion: AM had no effect on basal Leydig cell steroidogenesis but was inhibitory to HCG-stimulated and ET-1-stimulated testosterone production. Thus, AM may have a role in regulating Leydig cell steroidogenesis by interacting with HCG and ET-1. Correlated with this, AM and ET-1 were found to regulate the expression and secretion of each other in the isolated Leydig cells. AM inhibited the production of ET-1 while ET-1 augmented the production of AM. A similar relationship also exists in the Sertoli cells. The results showed that testicular AM and ET are inter-related at both the regulatory and the functional levels and together they may provide an important local mechanism for the fine-tuning of testicular functions. This study was supported by a grant from the Research Grant Council of Hong Kong (HKU7451/04M).

P41 Adrenomedullin and its receptors in the testis

Wai-Sum O, Yuk Yin

Faculty Of Medicine, The University Of Hong Kong, 21 Sassoon Road, Pokfulam, Hong Kong

Introduction: Adrenomedullin (AM), a hypotensive peptide, has been found in a large number of organs in the human and the rat. The aim of the present study is to find out whether or not AM and its receptors are expressed in the rat testis.

Methods: Adult Sprague-Dawley rat testes were used in all the experiments. AM was localized in the testis by immunocytochemical procedure. Testicular extract was used to measure the AM levels and RT-PCR was employed for the determination of mRNA contents of preproAM, the calcitonin receptor-like receptor (CGRP) and its activity modifying proteins (RAMP-1, -2 and -3).

Results & Discussion: Immunocytochemical staining showed positive AM immunostaining in the Leydig cells, Sertoli cells and myoid cells in the rat testis. Whole testicular extracts had 5.43 +/- 0.42 fmol of immunoreactive AM per mg of protein and 84 +/- 8 fg preproAM mRNA per pg β-actin mRNA. Gel filtration chromatography of AM showed two peaks with the predominant one eluting at the position of AM precursor. Scatchard plot analysis demonstrated specific binding of iodinated AM to the testis. Furthermore, the testis was shown to co-express mRNAs encoding the calcitonin receptor-like receptor (CGRP) and all three subtypes of receptor activity-modifying proteins. These account for the specific binding of AM in the testis, which was partially inhibited by human AM (22-52) and by human CGRP (8-37), the AM and CGRP antagonists respectively. Administration of AM to testicular blocks in vitro resulted in a dose-dependent inhibition of hCG-stimulated release of testosterone, which is abolished by the administration of AM (22-52). Our results strongly suggest a paracrine effect of AM on testicular steroidogenesis.

This study was supported by a grant from the Research Grant Council of Hong Kong (HKU7451/04M) and a Merit Award, the University of Hong Kong.

P42 IGF expression in bovine oviduct during negative energy balance

Mark Fenwick[1], Richard Fitzpatrick[2], David Kenny[3], D Claire Wathes[1]

[1]*Royal Veterinary College, London, UK*

[2]*Teagasc, Athenry, Galway, Ireland*

[3]*Faculty of Agriculture, University College, Dublin, Ireland*

Introduction: The growth hormone-IGF axis maintains nutritional homeostasis. In high yielding dairy cows systemic IGF levels decline in early lactation; a response which has lasting effects on fertility. This study determined whether energy balance status alters the pattern of IGF synthesis and regulation in the oviduct, which may adversely affect early stages of embryo growth.

Methods: Multiparous Holstein cows were allocated to 2 treatments (each n=6) designed to produce either mild (MNEB) or severe negative energy balance (SNEB). MNEB cows were fed *ad lib* grass silage supplemented with 8 kg day^{-1} concentrate and milked x1 daily. SNEB cows were restricted to 25 kg day^{-1} silage with 4 kg day^{-1} concentrate and milked x3 daily. Animals were culled in week 2 of lactation, a blood sample was taken and oviducts were collected for RNA analysis by real time RT-PCR and *in situ* hybridisation (ISH). Quantitative differences in oviduct gene expression were measured for all members of the IGF family (IGF-I/II, IGF binding proteins (BPs) -1-6 and receptors for IGF type 1 and 2, insulin A/B and GH).

Results & Discussion: SNEB cows had significantly reduced systemic concentrations of IGF-I and glucose (both $P<0.01$) and increased non-esterified fatty acids (NEFA; $P<0.01$) and β-hydroxybutyrate (BHB; $P<0.01$). By real time RT-PCR analysis, SNEB cows also had reduced expression of IGFBP-2 and -6 mRNAs ($P<0.05$ respectively), accompanied by elevated IGFBP-5 ($P<0.05$) when compared with MNEB cows. These effects were corroborated by ISH using optical density measurements for IGFBP-2 ($P<0.05$) and -6 ($P<0.01$) but not -5 ($P=0.447$). All other components measured remained unchanged between groups. When data from all animals was pooled, IGFBP-2 mRNA expression in the oviduct correlated positively with mean plasma glucose ($r=0.692$; $P<0.05$) and negatively with BHB ($r=-0.678$; $P<0.05$). Conversely, IGFBP-5 decreased with mean plasma glucose ($r=-0.734$; $P<0.02$) and increased with BHB ($r=0.727$; $P<0.05$). These results highlight a unique pattern of IGFBP expression associated with NEB, which may affect IGF bioavailability and oviduct function.

Funded by the Wellcome Trust.

P43 Expression of the IGF system in the involuting bovine uterus

Samantha Llewellyn[1], Richard Fitzpatrick[2], David Kenny[3], Martin Sheldon[1], Claire Wathes[1]

[1]*Department of Veterinary Basic Sciences, Royal Veterinary College, Potters Bar, Herts, LA9 7TA*

[2]*Teagasc Research Centre, Athenry, Co. Galway, Ireland*

[3]*Faculty of Agriculture, University College, Dublin, Ireland*

Introduction: Dairy cows enter a period of negative energy balance (NEB) in the early *post partum* period, which has both local and systemic effects on the IGF system. This period coincides with the process of uterine involution. We hypothesized that involution is delayed by NEB via effects on the uterine insulin-like growth factor (IGF) system.

Methods: Multiparous Holstein-Friesian cows (n=12) were randomly allocated to mild (mNEB) or severe negative energy balance (sNEB) groups. mNEB cows were fed *ad lib* grass silage + 8 kg day^{-1} concentrates and milked x1 daily; sNEB cows were fed 25 kg day^{-1} silage + 4 kg day^{-1} concentrate and milked x3 daily. At slaughter, 2 weeks after calving, blood was collected to measure hormones and markers of tissue mobilisation and tissue inflammation, whilst uteri were collected for localization of the IGF system by *in situ* hybridization. Relative expression in different uterine regions was measured as optical density units by image analysis.

Results & Discussion: Cows in sNEB had reduced ($P<0.05$) IGF-II mRNA expression in endometrial stroma (ES) and myometrium and mRNA expression was negatively correlated with plasma urea ($r = -0.747, -0.671$ respectively; $P<0.05$). The expression of IGFBP-2 and -4 mRNA increased ($P<0.05$) in sNEB, IGFBP-2 in the ES and IGFBP-4 in the intercaruncular subepithelial stroma (SES). This IGFBP-2 expression was positively correlated with circulating NEFA ($r = 0.720$; $P<0.01$). IGFBP-6 mRNA expression in the SES was decreased by sNEB ($P<0.01$), and negatively correlated with plasma NEFA ($r = -0.707$; $P<0.05$) and BHB ($r = -0.745$; $P<0.01$), but positively correlated with plasma glucose and oestradiol ($r = 0.677$ and 0.641, respectively; $P<0.05$). IGFBP-3 mRNA expression in the luminal epithelium and IGFBP-5 in the SES were both correlated to the serum amyloid A concentration ($r = 0.736$; $P<0.01$; and $r = -0.749$; $P<0.05$, respectively). In conclusion, gene expression for the IGF system in the involuting uterus was altered in relation to metabolic indices of tissue mobilization and inflammation. This may influence the rate of uterine involution and subsequent return to fertility.

P44 Effect of bacterial contamination in the postpartum bovine uterus

Aleisha Miller[1], Shan Herath[1], Clare Bryant[2], Martin Sheldon[1]

[1]*Royal Veterinary College, University of London, Department of Veterinary Clinical Sciences, UK*

[2]*University of Cambridge, Centre for Veterinary Science, Department of Veterinary Medicine, Madingley Road, Cambridge, CB3 0ES, UK*

Introduction: Bacterial contamination is ubiquitous in the bovine uterus after parturition, causing infertility. The most pathogenic organism in the uterus is *Arcanobacterium pyogenes*, as it is associated with disease severity. The uterine endometrium comprises of epithelial and stromal cells that secret prostaglandins (PG) and are the first line of defence against infection. The risk of uterine infection is greater during the luteal phase of the oestrous cycle than the follicular phase. Moreover, infection during the luteal phase often delays luteolysis. This study tested the hypothesis that *A. pyogenes* modulates PG production by the endometrium and purified populations of epithelial and stromal cells during the luteal phase.

Methods: Tissue explants, epithelial and stromal cell populations (n=3) were isolated from the uterine endometrium of cross breed heifers during the luteal phase. Endometrial explants or cells were stimulated for 24 hours in the presence of bacteria free filtrate (BFF) cultivated from *A. pyogenes* or heat-killed *A. pyogenes* (HKAP). Culture supernatants were collected for measurement of $PGF_{2\alpha}$ and PGE_2 by RIA.

Results & Discussion: Endometrial explants secreted both $PGF_{2\alpha}$ and PGE_2, whilst epithelial and stromal cells secreted $PGF_{2\alpha}$ and PGE_2, respectively when stimulated with *A. pyogenes*. Endometrial explants and epithelial cells stimulated with BFF secreted more $PGF_{2\alpha}$ than controls (12.8 +/- 2.4 vs 4.3 +/- 0.9 ng/ml, P<0.05; and 20.1 +/- 3.6 vs 8.9 +/- 1.7 ng/ml, P<0.05; respectively). Stromal cells secreted less PGE_2 than controls when treated with BFF (11.9 +/- 2.2 vs 22.1 +/- 1.8 ng/ml, P<0.05). In addition, tissue explants, epithelial and stromal cells stimulated with HKAP secreted $PGF_{2\alpha}$ in a dose response manner. In conclusion, *A. pyogenes* modulates the secretion of $PGF_{2\alpha}$ and PGE_2 by bovine endometrial cells. These changes in endometrial PG secretion associated with A. *pyogenes* may disrupt luteolysis and contribute to the infertility associated with uterine infection.

P45 Uterine cytokine arrays in the oestrous mouse

Nicolas Michel Orsi, Uma Vijaykumar Ekbote, James Johnston Walker, Nadia Gopichandran

Perinatal Research Group, Leeds Institute of Molecular Medicine, Level 4, JIF Building, St James's University Hospital, Beckett Street, Leeds LS9 7TF, UK.

Introduction: While cytokines regulate immune effector cell function in mammalian reproduction, they are also increasingly recognized as embryotrophins which may prove useful in embryo culture. However, little is known about their physiological profiles in the reproductive tract. This study profiled 23 cytokines (listed below) in uterine luminal fluid and serum in oestrous female CD1 mice.

Methods: Uterine fluid (collected under mineral oil) and serum (from blood collected by cardiac puncture) were analyzed by fluid-phase multiplex immunoassay. Differences between these were determined using Student's t-tests or Mann-Whitney-*U* tests, as appropriate.

Results & Discussion: The most pronounced differences between uterine fluid and serum were noted for eotaxin, interleukin (IL)-6, IL-1α, keratinocyte-derived chemokine (KC) and granulocyte-colony stimulating factor (G-CSF) (7, 13, 66, 165 and 173-fold higher in uterine fluid, respectively; P<0.001). While IL-1β (P<0.01), IL-2 (P<0.05), IL-3, IL-4 (P<0.01), IL-9 (P<0.05), granulocyte macrophage-colony stimulating factor, macrophage inflammatory protein (MIP)-1α (P<0.001), MIP-1β (P<0.05) and regulated upon activation, normal T-cell expressed and secreted RANTES (P<0.001) levels were also higher in uterine fluid (2, 2, 2, 2, 2, 3, 3, 2 and 6-fold, respectively), serum had higher profiles of IL-12 (p40) (P<0.001), IL-12 (p70) (P<0.01), IL-17 and interferon-γ (P<0.001) (2, 2, 24 and 4-fold, respectively). No significant differences in IL-5, IL-10, IL-13, monocyte chemoattractant protein-1 and tumour necrosis factor-α profiles were noted. These striking differences indicate a rigid compartmentalization of systemic and uterine cytokine-leukocyte networks. Uterine IL-1α, IL-6, G-CSF, KC, eotaxin and RANTES are proposed to regulate the recruitment, relocation and activation of eosinophils, neutrophils and macrophages essential for post coital processing of seminally-derived paternal antigens and the induction of uterine immunopermissiveness in preparation for blastocyst implantation. While they may further regulate endometrial angiogenesis, apoptosis, proliferation, and differentiation, they are good candidates to test for their embryotrophic properties.

P46 Effect of oxytocin addition on post thaw semen quality and fertility in Nili-Ravi buffaloes (Bubalus bubalis)

Muhammad Amir Saeed

Department of Theriogenology, University of Veterinary and Animal Sciences, Lahore-54000, Pakistan

Introduction: The objective of this study was to determine if addition of oxytocin has beneficial effect on post thaw semen quality and fertility rate in Nili-Ravi buffaloes.

Methods: Semen ejaculates from 2 buffalo bulls were pooled (n=4) and after preliminary evaluation diluted in tris-citric acid extender. Final volume was divided into 4 equal parts (A, B, C and D) and supplemented with oxytocin (Cintocinon® , Novartis, Pakistan) @ 2.5, 5.0, and 7.5 I.U/100mL of diluted semen with an untreated control group at 37°C. Semen was cooled slowly in 2 h from 37°C to 4°C, equilibrated at 4°C for 4 h, filled in 0.5 mL straws and frozen in liquid nitrogen (-196°C). After 24 h of deep-freezing, frozen semen was thawed at 37°C for 15 s and subjected to visual motility assessment (VM; %), plasma membrane integrity (HOS Assay; %), acrosome integrity (NAR; %) and viability (Live/Dead; %). Twenty-five straws from each treatment group were used for fertility trial. Data were presented as mean ± SEM.

Results & Discussion: Statistical analysis revealed a significant (P<0.05) difference in visual motility (%) amongst four treatments (46.67±1.67, 31.67±1.67, 50.0±2.89 and 56.67±1.67), respectively. HOS positive spermatozoa (%) were significantly (P<0.05) high in treatment C (28.87±1.03) than treatments A, B and D (19.75±1.36, 21.00±0.97 and 23.62±3.07), respectively. Live spermatozoa (%) were significantly (P<0.05) less in treatment B (56.00±0.81) than treatments A, C and D (65.5±0.28, 64.75±1.03 and 62.87±0.42), respectively. Acrosomal integrity did not differ significantly (P>0.05) amongst four treatments (76.87±0.68, 75.62±1.82, 81.00±1.14 and 78.37±3.37), respectively. Chi-square statistics revealed a significant high fertility rate (%) for treatment B (72) than treatments A, C and D (56, 40 and 44), respectively. This study revealed inconsistent effect on post thaw semen quality parameters, however, 0.5 I.U of oxytocin per 100mL of diluted semen resulted in improved fertility rate in Nili-Ravi buffaloes.

P47 Progesterone modulates the effect of bacterial lipopolysaccharide on prostaglandin secretion by uterine epithelial cells

Martin Sheldon[1], Robert O Gilbert[2]

[1]*Royal Veterinary College, University of London, UK*

[2]*Cornell University, Ithaca, USA*

Introduction: Endometrial epithelial cells are the first line of defence against bacterial infection of the uterus, as well as secreting prostaglandin E_2 (PGE) and $F_{2\alpha}$ (PGF), which regulate corpus luteum lifespan. The risk of infection is greater during the luteal than the follicular phase of the ovarian cycle, and infection often disrupts luteolysis. Epithelial cells express Toll-like Receptor 4 (TLR4), which detects lipopolysaccharide (LPS), the principal pathogenic moiety of the common uterine pathogen *Escherichia coli.* This study tested the hypothesis that LPS and progesterone modify the prostaglandin secretion of endometrial cells.

Methods: Epithelial cells from murine and bovine endometrium were cultured with luteal phase concentrations of progesterone (10 ng/ml) or vehicle and 18h later challenged with *E. coli* LPS (10 μg/ml). After a further 24h, supernatants were collected for PGE and PGF measurement by ELISA.

Results & Discussion: When challenged with LPS, murine epithelial cells secreted more PGE compared with controls (7.9 +/- 0.1 vs 7.1 +/- 0.1 ng/ml; $P < 0.05$) and less PGF (23.1 +/- 3.9 vs. 62.2 +/- 3.6 ng/ml; $P < 0.05$). The LPS challenged bovine cells also secreted more PGE (5.5 +/- 0.6 vs 1.7 +/- 0.4 ng/ml; $P < 0.05$), although PGF secretion was not affected. This LPS-induced PGE secretion was suppressed by progesterone in murine (1.7 +/- 0.6 ng/ml; $P < 0.05$) and bovine epithelial cells (2.3 +/- 0.4 mg/ml; $P < 0.05$). Progesterone abrogated the PGF secretion by murine cells ($P < 0.05$) but had little effect on the bovine cells. The increased ratio of PGE:PGF secretion by endometrial epithelial cells following LPS challenge may disrupt luteolysis, whilst the reduced prostaglandin secretion in the presence of progesterone may suppress the immune response. In conclusion, LPS modifies the endometrial epithelial cell prostaglandin secretion and this response is modulated by progesterone.

IMS was an OECD Fellow.

NOTES

With thanks to the following sponsors:

Ferring Pharmaceuticals and QIAGEN

Conference Secretariat:
World Event Management, Summit House, Woodland Park, Cleckheaton BD19 6BW
Tel: +44 (0)1274 854100 Fax: +44 (0)1274 854110 Email: srf2006@world-events.com www.srf-reproduction.org