SOCIETY FOR REPRODUCTIVE BIOLOGY

PROGRAM AND ABSTRACTS

Forty-Second
Annual Scientific Meeting

7th - 9th October 2011

CAIRNS CONVENTION CENTRE, AUSTRALIA

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Cover Image Description:
The cover image is of a bundle of approximately one hundred Echidna spermatozoa. Monotreme sperm bundle together in this fashion in the female reproductive tract as a competitive advantage to reach the oocyte.
The sperm are labelled with Nucleus DAPI (blue), Acrosome PNA (green) and Tail Tubulin (red).
Image courtesy of Amanda Anderson, University of Newcastle.
For more information on monotreme fertility see SRB oral presentation by Brett Nixon Sunday 9th Oct Abstract # 49 B NIXON.

SRB OFFICE BEARERS 2011

President Mark Hedger (2010-onwards)
Secretary Jane Girling (2011- onwards)
Treasurer Richard Nicholson (2010 - onwards)
POC co chair Eileen McLaughlin
POC co chair Charles Allan
Membership Larry Chamley
Sponsorship Wendy Ingman
Public Relations Sarah Meachem
Ordinary Kirsty Pringle

Communication Officer David Sharkey
Postdoc Representatives Adam Koppers and Natalie Hannan
Postgraduate Reps Matt Dun and Chez Viall
Newsletter Jemma Evans
WCRB POC Chair Darryl Russell
Awards Committee Chair Eva Dimitriadis

PAST SRB OFFICE BEARERS

Year	Chairperson	Treasurer	Secretary
1969 - 70	TJ Robinson	RG Wales	JR Goding
1970 - 71			
1971 - 72			
1972 - 73		IG White	BM Bindon
1973 - 74	CW Emmens		
1974 - 75			
1975 - 76			
1976 - 77		GM Stone	IA Cumming
1977 - 78	DM de Kretser		
1978 - 79			
1979 - 80			
1980 - 81		GH McDowell	RJ Scaramuzzi
1981 - 82	NW Moore	BG Miller	
1982 - 83			TK Roberts
1983 - 84	JK Findlay		
1984 - 85		CD Nancarrow	JM Cummins
1985 - 86	BM Bindon		
1986 - 87			G Evans
1987 - 88	BP Setchell	LA Hinds	
1988 - 89			JM Shelton
1989 - 90	JC Rodger	GB Martin	
1990 - 91			PL Kaye
1991 - 92	RJ Scarammuzzi	CG Tsonis	
1992 - 93			LA Salamonsen
1993 - 94	AO Trounson	J Wilton	
1994 - 95			MP Hedger
1995 - 96	RF Seamark	JD Curlewis	
1996 - 97			JG Thompson
1997 - 98	MB Renfree	MB Harvey	
1998 - 99			
1999 - 00			T Lavranos
2000 - 01	JP Hearn	C O'Neill	
2001 - 02			RB Gilchrist
2002 - 03			
2003 - 04	LA Salamonsen	KL Loveland	
	President		
2004 - 05	LA Salamonsen		C Gargett
2005 - 06			
2006 - 07	M Holland	SA Robertson	
2007 - 08			C. Grupen
2008 - 09			
2009 - 10	M Hedger	R Nicholson	
2010 - 11			J Girling

CONFERENCE ORGANISING COMMITTEES

The Local Organising Committee SRB/WCRB
Darryl Russell, Charles Allan, Eileen McLaughlin, Eva Dimitriadis, Mark Hedger and Guiying Nie (ANZPRA)

SRB Program Organising Committee
Charles Allan and Eileen McLaughlin (Co-chairs)
Darryl Russell, Eva Dimitriadis, Claire Roberts, Shaun Roman and Ellen Menkhorst
The Program Committee thanks those members who assisted in reviewing the abstracts.

Conference and Society Secretariat
ASN Events Pty Ltd
3056 Frankston-Flinders Road, (PO Box 200), BALNARRING VIC 3926
Phone: 03 5983 2400 Fax: 03 5983 2223 Email: mp@asnevents.net.au

AWARD WINNERS

Year	Goding / Founders Lecture	JSA / New Investigator Award	SRB / RCRH Award	MLA New Scientist Award	TJ Robinson Award (4 year)
1974 - 75	IA Cumming				
1975 - 76	W Hansel				
1976 - 77	DT Baird				
1977 - 78	TD Glover				
1978 - 79	CH Tyndale-Biscoe				
1979 - 80	GMH Thwaites				
1980 - 81	KP McNatty				
1981 - 82	BK Follett	PJ Lutjen			
1982 - 83	J Wilson	RJ Rodgers & CB Gow			
1983 - 84	BM Bindon	SP Flaherty			
1984 - 85	A Bellvé	C O'Neill			
1985 - 86	BP Setchell	BJ Waddell			
1986 - 87	W Hansel	LJ Wilton			
1987 - 88	HG Burger	A Stojanoff			
1988 - 89	FW Bazer	MB Harvey			
1989 - 90	GD Thorburn	AH Torney			
1990 - 91	RM Moor	H Massa			
1991 - 92	CR Austin	DK Gardner			
1992 - 93	JK Findlay	SW Walkden-Brown			
1993 - 94	GC Liggins	CM Markey			
1994 - 95	I Huhtaniemi	MJ Hötzel, S McDougall			
1995 - 96	RF Seamark	I van Wezel			
1996 - 97	*No Lecture*	S Robinson			
1997 - 98	F Bronson	MJ Jasper			
1998 - 99	DM de Kretser	M Panteleon			
1999 - 00	I Wilmut	E Whiteside			
2000 - 01	R Short	CE Gargett			
2001 - 02	D Albertini	WV Ingman			
2002 - 03	AO Trounson	J Smith			CG Grupen
2003 - 04	T Fleming	AN Sferruzzi-Perri			
2004 - 05	RJ Aitken	K Webster		T Hussien	
2005 - 06	J Eppig	T Hickey		M Herrid	
2006 - 07	M Renfree	K Walters	M O'Bryan	P Hawken, Z Zhang	KM Morton
2007 - 08	M Matzuk	C Hogarth	E McLaughlin	KH Beilby	
2008 - 09	J Robinson	G Wilson	SA Robertson	T Flatscher-Bader	
2009 - 10	LA Salamonsen	AS Care	RB Gilchrist	M Bertoldo	
2010 - 11	R R Pera	P Nichols	E Dimitriadis		

PRINCIPAL GUEST SPEAKERS FOR 2011

FOUNDERS LECTURER

Chris O'Neill
Centre for Developmental and Regenerative Medicine
University of Sydney, NSW, Australia

Chris obtained both his B.Sc. and PhD from the Department of Biological Sciences, University of Newcastle. After a brief period of post-doctoral work in the Vet School at Sydney University, he moved to the fledgling IVF program at Royal North Shore Hospital in 1984. He helped develop this program, becoming its Director in the early 1990s. Upon the move of the program to the commercial section, Chris joined the Department of Physiology, University of Sydney.
He is currently Associate Professor in the University's Departments of Medicine and Physiology; Head, Division of Developmental and Regenerative Medicine, Kolling Institute for Medical Research; and Co-Leader - Reproductive, Maternal and Child Health Theme, Sydney Medical School.

PRESIDENT'S LECTURER

Ray Rodgers
Professor, Deputy Director of the Robinson Institute,
School of Paediatrics and Reproductive Health,
University of Adelaide, SA, Australia

Ray Rodgers has a B.Agr.Sci., M.Agr.Sci. and PhD from the University of Melbourne and undertook a postdoctoral fellowship at the University of Texas Southwestern Medical School Dallas. He has held QEII fellowship and NHMRC fellowships and his research is funded by Program and Project grants from the NHMRC and the ARC. In his early years he made major contributions to the study of luteal cells and steroidogenic enzymes in the ovary. Then in the early 1990's he established a group examining the roles somatic stem cells and extracellular matrix in ovaries. They discovered a novel extracellular matrix, called focimatrix, and are actively deciphering its role in maturation of granulosa cells. They have also identified how another extracellular matrix protein, fibrillin 3, could be involved in the aetiology of PCOS. Major activities of Ray Rodgers include receiving editor of Molecular and Cellular Endocrinology since 2000, member of the NHMRC Academy and past president of the Endocrine Society of Australia.

SYMPOSIA SPEAKERS

Mats Brännström
University of Gothenburg, Sweden

Mats Brännström is professor and chairman of Department of Obstetrics and Gynecology , University of Gothenburg, Sweden. He completed his PhD in Physiology in 1988, with a thesis on Mechanisms of Ovulation. After further research training at University of Miami and a 3-year post doc period at University of Adelaide he completed his clinical training in Obstetrics and Gynecology, subspecialising in gyne-oncology surgery. His research group is currently involved in research on ovulation, ovarian cryopreservation, PCOS and uterus transplantation.

Paul Fowler
University of Aberdeen, United Kingdom

Paul Fowler is Professor of Translational Medicine at the Institute of Medical Sciences at the University of Aberdeen. Over the last decade he has focused on fetal human development and the effects of environmental chemicals and endocrine disrupters on reproductive development in humans and model species. A major interest in this field is the effects of long-term, real-life exposures to complex chemical cocktails on fetal development and adult reproductive capacity. Current activities include coordination of a €2.9 million European Commission project "Reproductive Effects of Environmental Chemicals in Females" as well as studies of retinoic acid and endocannabinoid pathways in the human fetal gonad. Recent key publications include the first demonstration of a fetal human testis gene disturbed by maternal cigarette smoking and the first gene array based investigation of human fetal primordial follicle formation. Paul is Chair of the Society for Reproduction & Fertility and a member of the standing evaluation panel on Environmental Pollution for the Swedish Research Council Formas.

Phil Knight
University of Reading, United Kingdom

Phil Knight graduated in Zoology from UCNW Bangor in 1976 and completed an MSc in Radiobiology at Birmingham University before moving to Reading University in 1977 for a PhD on the role of hypothalamic catecholamines in the regulation of avian gonadal function, supervised by Frank Cunningham and Dick Gladwell. He stayed there as a post-doc before taking a temporary lectureship to cover for the late Geoff Waites during his secondment to WHO in Geneva. He gained a full Lectureship in 1984, Readership in 1993 and Personal Chair in 1998. From 2003-2005 he was head of the School's Cell and Molecular Biology Division. His involvement with Geoff Waite's group back in 1983 introduced him to the delights of domestic ruminant research and since that time his work has encompassed both mammalian and avian reproduction with his research focus gradually shifting from hypothalamic-pituitary interactions to pituitary-ovarian and intraovarian interactions. He has published 113 full papers and 16 invited reviews/book chapters and has supervised 17 PhD students.

Eileen McLaughlin
Priority Research Centre in Reproductive Science
School of Environmental & Life Sciences University of Newcastle, NSW, Australia

Professor Eileen McLaughlin is a PhD graduate of the University of Bristol, UK (1994) and now Deputy Head of School and Co-Director of the Priority Research Centre for Chemical Biology, School of Environmental & Life Sciences, University of Newcastle. Her current research is concentrated in two main areas. In spermatogenesis, she is working on a number of key molecules which contribute to the control of germ cell cycle and regulation of meiosis in the testes. In folliculogenesis, she has focussed on cell signalling pathways that contribute to the initiation of primordial follicle, effects of environmental ovotoxicants and development of nonsurgical sterilant agents for domesticated animals. Similarly, her work on the Musashi family of RNA binding proteins and the cell cycle regulator APCfzr1 in meiosis in the testes has been recognised by invitations to present her work at the Cold Spring Harbor Germ Cell Meeting, OzBio and Hunter Cell Biology meetings in 2010. Her work is funded by Project grants from the NHMRC, Found Animals, CSIRO and the ARC. In 2007 she was awarded the Society for Reproductive Biology RCRH Award for Excellence in Reproductive Biology Research and in 2010 was appointed to a personal chair in Biological Sciences at Newcastle. Her most recent research on ovarian follicular development led to her invitation to present in the recent Serono Symposia International Foundation conference on: "Reproductive ageing - a basic and clinical update" in Sicily in April 2011.

Moira O'Bryan
Department of Anatomy and Developmental Biology
Monash University, VIC, Australia

Moira O'Bryan graduated from The University of Melbourne in 1994, after which she was awarded an Andrew Mellon Foundation Fellowship to work at The Population Council (at Rockefeller University) in New York. She returned to Australia on a National Health and Medical Research Council (NHMRC) Peter Doherty Fellowship in 1996 to work at Monash Institute of Medical Research (nee Monash Institute of Reproduction and Development), Monash University where she established a highly productive lab working on sperm development and the genetics of male infertility. Moira was subsequently awarded a NHMRC R.D. Wright Fellowship, a Monash University Senior post-doctoral Fellowship from the Faculty of Medicine and two NHMRC Senior Research Fellowships. In 2009 Moira and her lab moved to the Department of Anatomy and Developmental Biology where she heads the "Male Infertility and Germ Cell Biology Laboratory" and is Deputy Head of Department. The focus of her current research is on the identification of novel regulators of male fertility, CRISP / CAP proteins and the causes of human male infertility. Moira has received research based awards from the Australian Academy of Science, The Fertility Society of Australia, The Endocrine Society of Australia, and the Society for Reproductive Biology from whom she received the 2006 Research Centre for Reproductive Health Award for Excellence in Reproductive Biology. In 2008 she was named by the American Society of Andrology as "The Young Andrologist of the Year" and in 2010 she received the Dean's Award for Research Excellence from Monash University. In addition to a strong commitment to research excellence, Moira has achieved a substantial track record for the promotion of science through her position as a national director of The Australian Society for Medical Research (2003-2005). She has also made significant contributions to the infrastructure of Australian medical research through the establishment of The Australian Phenome Bank and the Australian Centre for Vertebrate Mutation Detection, the Monash Male Infertility Repository and the Australian Phenomics Network. Currently Moira is a member of the 'Male Infertility Advisory Board' of Andrology Australia and the management group of 'Australian Phenomics Network'.

Sarah Robertson
Professor, School of Reproductive Health and Paediatrics
University of Adelaide, SA, Australia.

Professor Sarah Robertson is a PhD graduate of the University of Adelaide, Australia (1993) and now NHMRC Principal Research Fellow and Co-Director of the Research Centre for Reproductive Health, Robinson Institute, University of Adelaide. Her research focus is the biology of conception and early pregnancy, and the immunology and pathophysiology of infertility and miscarriage. She has defined how the female immune system acts to exert 'quality control' in the reproductive process and how male seminal fluid signals to the female reproductive tract at coitus to influence conception and implantation. Her research has led to a novel approach to IVF embryo culture, utilising growth factors present naturally in the mother's uterus. Her work is funded by Program and Project grants from the NHMRC and the ARC. Since 2009 she has been Editor-in-Chief of the Journal of Reproductive Immunology (Elsevier, Ireland).

Darryl Russell
Ovarian Cell Biology Laboratory, Robinson Institute, University of Adelaide, SA, Australia

Dr Darryl Russell is head of the Ovarian Cell Biology Laboratory in the Robinson Institute and co-director of the Research Centre for Reproductive Health, University of Adelaide. He obtained his PhD from Monash University under Jock Findlay and postdoc training with JoAnne Richards at Baylor College of Medicine. He currently holds an ARC Future Fellowship and several research project grants. His group focuses broadly on cell-cell and cell-matrix interactions and tissue morphogenesis in normal function and cancer of the reproductive organs. He directs research focussed on the cell biology of ovarian cells including the function of cumulus and granulosa cells in folliculogenesis and oocyte maturation, mechanisms of ovulation and intercellular communication between ovarian cells and oocytes. Through these projects he is generating new basic knowledge and clinical tools to improve the production and identification of high developmental competence oocytes.

Justin St John
Professor and Director
Centre for Reproduction & Development
Monash Institute of Medical Research, Vic, Australia

Professor Justion St John was appointed Director of the Centre for Reproduction and Development, MIMR in November 2009. Prior to that, he held the position of Professor of Reproductive Biology, at the Mitochondrial and Reproductive Genetics Research Group, Clinical Sciences Research Institute, Warwick Medical School, University of Warwick. He attained his PhD from the University of Birmingham, UK and then undertook postdoctoral training as a Mellon Fellow at Oregon Regional Primate Research Center, USA before returning to the University of Birmingham to take up a lectureship in Mitochondrial and Reproductive Genetics. He was recently a recipient of an Endeavour Award that allowed him to undertake two periods of research at MIMR between 2007 and 2008. His work primarily focuses on how mitochondrial DNA is transmitted and replicated as it passes from the oocyte and embryo through to the offspring and differentiated embryonic stem cells so that they can meet their requirements for ATP derived through oxidative phosphorylation. In order to understand the importance of the regulation of mtDNA transmission and replication in the embryo and how this impacts on embryonic stem cell viability, he has used models that tightly regulate this process, such as in vitro fertilization, and those that violate this regulation, for example somatic cell nuclear transfer. He is now applying these outcomes to determine whether pluripotent stem cells derived through somatic cell-embryonic stem cell fusion and induced pluripotency are able to acquire the tight control of mtDNA replication effectively to mediate cellular function once they have fully committed to specific fates.

Hongshi Yu
University of Melbourne, Vic, Australia

Dr. Hongshi Yu was awarded Ph.D. in molecular and developmental genetics at Wuhan University in 2003. Since 2004, he has been at the University of Melbourne with Professor Marilyn B Renfree, studying reproduction and development using the tammar as a model supported by the NH&MRC and the ARC Centre of Excellence in Kangaroo Genomics. He is interested in the evolutionary conservation of the sex determining genes. He has investigated tammar HOX clusters and the role of long non-coding RNAs in the developing reproductive system and is currently using tammar sex reversal model to discover novel genes.

SRB Awards

SRB RCRH Award Winner

Caroline Gargett
The Ritchie Centre, Monash Institute of Medical Research and
Monash University Department of Obstetrics and Gynaecology, Vic, Australia

A/Prof Caroline Gargett, NHMRC RD Wright Fellow, is Deputy Director of The Ritchie Centre in the Monash Institute of Medical Research and heads the Women's Health Theme. She discovered epithelial progenitors and mesenchymal stem cells in the endometrium and featured in Australia's National Health and Medical Research Council's publication "10 of the Best Research Projects in 2008". She was recently honoured for her pioneering research in Endometriosis by the Endometriosis Foundation of America (2011). She serves on the Editorial Boards of Fertility and Sterility and Reproductive Sciences and was Associate Editor for Human Reproduction (2005-2008). She is Vice President of the Australasian Society for Stem Cell Research and Secretary for SRB (2005-2007).

Newcastle ECR Award Finalists

Catherine Itman
Monash Institute of Medical Research, Vic, Australia

Catherine Itman earned her doctorate from Monash University in 2008 and was awarded a Monash Bridging Fellowship to continue her research. Based at Monash School of Biomedical Sciences, she received a 2010 NH&MRC New Investigator Grant to investigate hormone, growth factor and endocrine disruptor actions on testis development and adult health. Catherine's awards include the 2010 Toshiya Yamada Early Career Award, 2009 American Society of Andrology (ASA) Outstanding Trainee Investigator Award and she was a 2009 SRB New Investigator finalist. In addition to research, her contributions include serving on University and society committees, chairing conference sessions, teaching and research training.

Jeremy Smith
Research Fellow, Monash University, Vic, Australia

Jeremy Smith began his research career at The University of Western Australia, completing his PhD – awarded Distinction– in 2004. In 2003, Jeremy was awarded a NICHD U54 Cooperative Contraceptive Research Centers Fellowship (US) and worked with Professor Robert Steiner at the University of Washington. Jeremy investigated kisspeptin, a novel neuropeptide, vital in the neuroendocrine control of GnRH secretion and reproductive function. In 2006, Dr Smith received a NHMRC Biomedical Fellowship and returned to Australia to the Department of Physiology, Monash University. Jeremy is further funded by an NHMRC project grant and is a recipient of a prestigious ARC Future Fellowship.

Ulla Simanainen
ANZAC Research Institute, NSW, Australia

Dr Simanainen is an early career scientist who was awarded her PhD from University of Kuopio, Finland, in 2004. Her research career started in the area of toxicology with the specific focus on male reproductive effects of dioxins. During her PhD she spent a year in the University of Wisconsin-Madison, USA as a Fulbright Fellow. After receiving her PhD she started as a post-doctoral fellow at the Andrology lab at ANZAC Research Institute in Sydney, March 2005. Her work has focused on detailing androgen actions in male and female sex accessory glands including prostate, epididymis and breast.

Stephen Tong
Mercy Hospital for Women, Vic, Australia

A/Prof Tong is a clinician-scientist at Mercy Hospital for Women. He leads The Translational Obstetrics Group, a laboratory with a keen focus on translational research; developing molecularly targeted approaches to treat pre-eclampsia and ectopic pregnancies, identifying blood-based biomarkers to identify fetuses critically hypoxic and developing biomarkers to predict pregnancy complications.
A/Prof Tong has obtained research grants totaling over 2 million dollars and published 39 papers, mostly in the top journals of his field. He has senior authorships in Nature (2002) and Lancet (1998 and 2002). In 2007 he received an NHMRC Achievement Award for the top ranked Clinical CDA Fellowship.

Important information for SRB students and early career researchers (ECRs)

Friday 7th October

5.00 - 7.00pm: **Early registration for the SRB meeting.**

7.00pm onwards: **Welcome drinks (Vortex)**. Drinks, canapés, students, ECRs and other delegates all vortexed together.

Saturday 8th October

8:30 – 10:00 am: **ANZPRA Award**. Given to ANZPRA member for the best oral **on placental biology** by a postgraduate student or early post doctoral researcher.

8:30 – 10:00 am: **Newcastle ECR Award**. Newly introduced SRB early career award to recognize an emerging and excellent contributor working in the field of Reproductive Biology – all four finalists selected from a very competitive panel of applicants – strong support from the ECR contingent is important.

1.30 – 3.00 pm: **SRB NIA award.** Given to the oral best presentation by a postgraduate student or early post doc researcher. Come and cheer along your fellow students at this prestigious and eagerly contested award session.

3:30 – 5:00 pm: **Meat and Livestock Australia and Oozoa Awards.** Given to the best oral presentation on reproduction in livestock by a postgraduate student/early post doc researcher (MLA) or on oocytes or sperm by a postgraduate student (Oozoa).

5.30 – 6.30 pm: **Annual student meeting.** This is a chance to air any issues you may have as an SRB student and to elect two new student representatives – so have a think about who you think would fit the job. SRB travel awards will also be presented during this meeting. You need to be present to collect your award.

5.30 – 6.30 pm: **Annual ECR meeting.** Talk to your ECR council member and elect two new ECR representatives – enthusiastic volunteers able to contribute to further the views and needs of ECRs are wanted for these positions.

7.00 – 11.00 pm: **SRB Dinner.** Food, drink and award presentations.

11.00 pm onwards: **Post-dinner student/ECR party.** For those of you who are still going after the SRB dinner. Venue TBA at the annual student/ECR meetings.

Sunday 9th October

7.30 – 8.30am: **Student mentor session.** This breakfast session (some, food is included!) is a must-attend for those of you who are already thinking about the next step; post-doctoral research. It is also an opportunity to gain insight from experienced investigators about topics that may relate to your Ph.D. Matt and Chez will co-chair discussion with 3 distinguished PI's to give you valuable advice pertaining to issues that affect you.

12.10 – 1.00 pm: **ECR mentor session.** This lunch-time session provides ECRs an opportunity to gain important advice regarding career development and discuss any issues that you may be facing with established researchers. We have fantastic prominent reproductive research scientists to provide invaluable advice and perspective to ECRs. Natalie Hannan will chair the session and encourage valuable discussion and advice pertaining to issues that affect you now and in the future. This lunch is a must for those of you early in your career. Grab you lunch and head straight to the meeting room (detailed in the program) to get the most out of this session.

6.30 – 7.30 pm: **Optional rugby viewing at the Welcome drinks for WCRB.** Watch the fourth RWC 2011 quarter final live from Auckland between the winner of pool A (likely to be the All-Blacks) and the runner-up of pool B. Venue TBA at the annual student meeting.

WANT TO BECOME AN SRB STUDENT MEMBER?

Membership gives you:

- Discounted registration at the SRB Annual Scientific Meeting
- Free SRB Newsletters
- Eligibility for SRB Travel Awards, the New Investigator Award and other awards sponsored at the Annual Meeting
- Reduced purchase price on journals: Reproduction Fertility Development and Biology of Reproduction
- Access to a professional network in the field of Reproductive Biology
- Eligibility for Affliate Membership of SSR
- Discounted student and 3yr memberships
- Eligibility for student positions within SRB

Email student reps (Chez – c.viall@auckland.ac.nz or Matt – Matt.Dun@newcastle.edu.au) and ECR reps (Natalie – nhannan@unimelb.edu.au or Adam – adam.koppers@monash.edu) if you have any suggestions, problems or queries regarding the meeting.

FUTURE MEETINGS

2011

The 14th World Congress on Human Reproduction
30th November – 3rd December 2011
Melbourne Exhibition & Convention Centre
http://www.humanreproduction2011.com/

2012

ESA – SRB Annual Scientific Meeting 2012
26th – 29th August 2012
Gold Coast Convention Centre
http://www.esa-srb.org.au/

INFORMATION FOR DELEGATES & PRESENTERS

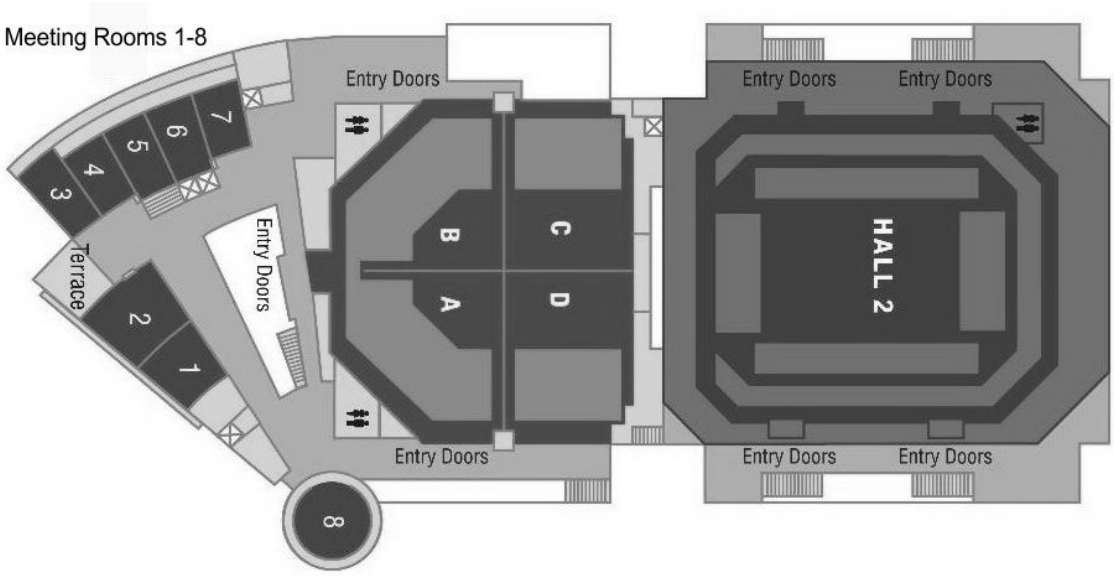
Venue
Cairns Convention Centre
Cnr Wharf & Sheridan Street
(PO Box 8084) Cairns 4870
Queensland, Australia
Phone: (07) 4042 4200

Registration

The Full SRB Delegate Registration fee includes:
* all delegate materials (name tag, satchel, abstract book)
* lunches (Saturday, Sunday)
* morning teas (Saturday, Sunday)
* afternoon teas (Saturday, Sunday)
* the welcome function

The Day Registration fee includes:
* all delegate materials (name tag, satchel, abstract book)
* lunch for the specified day
* morning tea for the specified day
* afternoon tea for the specified day

Venue Layout
The registration desk is located on the Groundfloor Foyer at the entrance. All sessions and breaks are to be located upstairs on the mezzanine level.



ORGANISER'S OFFICE AND REGISTRATION DESK
The organiser's office and registration desk will be located on the Groundfloor Foyer of the Cairns Convention Centre and open Saturday 8th October from 7:00 AM to 5:00 PM and on Sunday 9th October from 7:00 AM – 6:30 PM. A temporary desk will be open on the Friday night at the Cairns Sebel Hotel from 4pm.

Speaker Preparation
Presentations are to be loaded directly onto the PC located within the room that the talk will be given in, at least a full session in advance of your session. You should bring your talk on a USB, saved in a format for display on a PC within the room. A technician will be on hand to assist with any transfer / loading issues and to help you check your presentation. Please note there are no Macintosh computers in the presentation rooms, PC format only.

Name Tags
Delegates are required to wear their name tags to all scientific and catered sessions. Delegates should note that within their name tag pouch are any specific function tickets they have ordered. All those who are attending WCRB should retain their name tag for both meetings.

Email Access
Wireless access will be available and is free for all delegates in the Cairns Convention Centre. The Internet Café is in the Level 1, Mezzanine Foyer and will be open during conference hours.

Social Functions

- The **Welcome Function** is at the Cairns Sebel Hotel on Friday evening from 7:00pm. Light refreshments and drinks will be served and the function is complimentary for all registration types. The function will take place on level 2. Additional tickets for partners can be purchased from the registration desk.

- The **SRB Conference Dinner** will be held on Saturday evening at the Red Ochre Restaurant, Cairns. Pre-dinner drinks will be served from 7:00pm for a 7:30pm start. Dress is neat casual. **This is a ticketed function** and they must be purchased in advance from the registration desk.

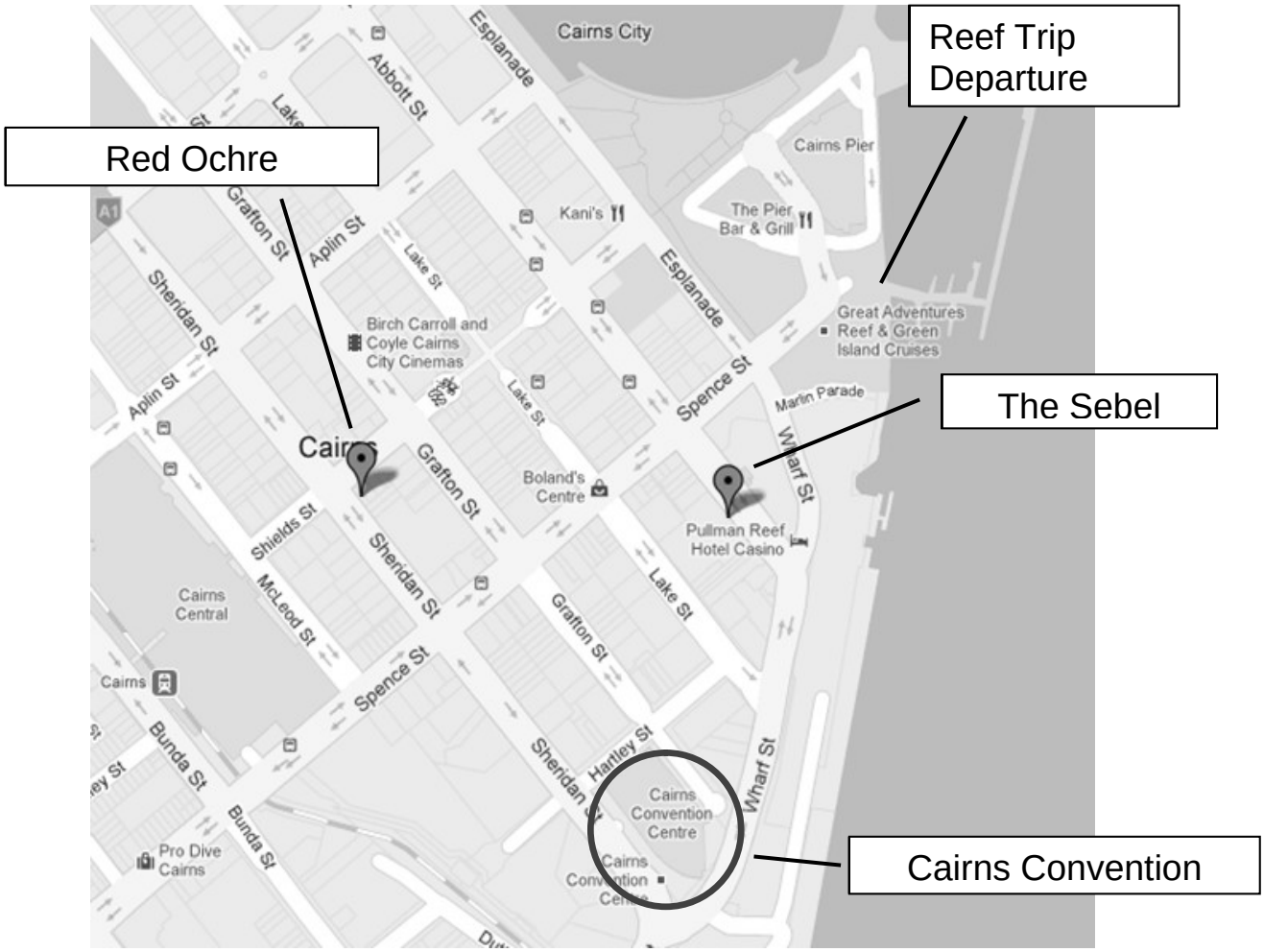
Insurance - The hosts and organisers are not responsible for personal accidents, any travel costs, or the loss of private property and will not be liable for any claims. Delegates requiring insurance should make their own arrangements.

Smoking - is not permitted in the venue.

Mobile Phones - Please ensure they are turned off during any session you attend.

Message Board - will be available at the registration desk.

Disclaimer - The hosts, organisers and participating societies are not responsible for, or represented by, the opinions expressed by participants in either the sessions or their written abstracts.



PROGRAM

Friday, 7 October 2011

Temporary Registration Desk Opens At Sebel
4:00 PM - 8:00 PM — Cairns Sebel Hotel - Conference floor

SRB Council Meeting
5:00 PM - 7:00 PM — Blue Water 2 – Cairns Sebel Hotel

SRB/ANZPRA Welcome Drinks
7:00 PM - 8:00 PM — Cairns Sebel Hotel - Conference floor

Saturday, 8 October 2011

Registration Desk Open
7:00 AM - 8:30 AM — Cairns Convention Centre - Ground floor foyer

Newcastle Reproductive Science Early Career Researcher Award
Chairs: John Aitken and Rick Nicholson

8:30 AM - 10:00 AM — Meeting Room 1+2

8:30am **Catherine Itman**
Activin-hormone crosstalk in testis development and dysgenesis: clues from Smad3 and Inhibin mice. *abs#001*

8:50am **Jeremy Smith**
Kisspeptin and reproduction: examining GnRH response in the median eminence and tracing axon terminals in the ovine brain. *abs#002*

9:10am **Ulla Simanainen**
Insights into androgen action from tissue-selective androgen receptor knockout mouse models *abs#003*

9:30am **Stephen Tong**
Combination Epidermal Growth Factor inhibition and methotrexate to medically treat ectopic pregnancies: bench to bedside *abs#004*

The SRB acknowledge the support of:

Australian and New Zealand Placental Research Association (ANZPRA) Award

Chairs: Claire Roberts and Larry Chamley

8:30 AM - 10:00 AM Meeting Room 3

8:30am **Georgia Kafer**
The role of histone variant h2afz in trophoblast differentiation: implications for the development of *in vitro* generated preimplantation mouse embryos *abs#005*

8:45am **Yu Wang**
Identification of the renin angiotensin system (RAS) pathways in BeWo and HTR-8/SVneo cells *abs#006*

9:00am **Megan Jones**
The effects of dietary Omega-3 fatty acids on inflammatory and oxidative status of the rat placenta *abs#007*

9:15am **Amanda Highet**
Low oxygen and cell-extracellular matrix interactions alter expression of invasion-modulating genes in the first trimester trophoblast cell line, HTR8/SVneo. *abs#008*

9:30am **Jessica Laurence**
Effect of dietary vitamin D and calcium deficiency on pregnancy status in C57Bl6 female mice *abs#009*

9:45am **Shane Sykes**
Plasma prorenin levels at 15 weeks gestation in women who had normal and complicated pregnancies *abs#010*

The SRB acknowledge the support of:

Morning Tea

10:00 AM - 10:30 AM Cairns Convention Centre - Upstairs foyer

Ovarian Function and Folliculogenesis

Chairs: Keith Jones and Rob Gilchrist

10:30 AM - 12:00 PM Meeting Room 1+2

10:30am **Darryl Russell**
Oocyte maturation and ovulation, coordination by the cumulus complex *abs#011*

11:00am **Kirsty Walters**
Sub-fertility in mice lacking functional androgen receptors in granulosa cells *abs#012*

11:15am **Kirsten McTavish**
FoxL2 is involved in GDF-9 and activin regulation of follistatin in granulosa cells: GCT FoxL2^{C134W} mutation exerts dominant activity *abs#013*

11:30am **Karla Hutt**
Loss of pro-apoptotic proteins, Puma and Noxa, prevents oocyte death and preserves fertility following anti-cancer therapy-induced DNA damage *abs#014*

11:45am **Janet Holt**
The APC/C activator FZR1 controls the timing of oocyte meiotic resumption in mammalian oocytes in a phosphorylation-dependent manner *abs#015*

Spermatogenesis and Testicular Function

Chairs: Charles Allan and Zamira Gibb

10:30 AM - 12:00 PM Meeting Room 3

10:30am **Eileen McLaughlin**
Mammalian meiosis: cell cycle regulation and male fertility *abs#016*

11:00am **Helen Abud**
The critical requirement for Wnt signalling in spermatogenesis *abs#017*

11:15am **Justine Olcorn**
The regulation of Sertoli cell micro-RNAs by TGFβ3 *abs#018*

11:30am **Andrew Major**
Paraspeckle protein 1 nuclear import is mediated by importin alpha 2; implications for testicular paraspeckle function *abs#019*

11:45am **Lisa Mitchell**
Phosphoinositide-3-kinase signaling pathway involvement in a truncated apoptotic cascade associated with motility loss and oxidative DNA damage in human spermatozoa *abs#020*

Lunch

12:00 PM - 1:30 PM Cairns Convention Centre - Upstairs foyer

SRB AGM

12:30 PM - 1:30 PM Meeting Room 3

SRB New Investigator Session

Chairs: Mark Hedger and Jane Girling

1:30 PM - 3:00 PM Meeting Room 1+2

1:30pm **Laura Frank**
Proteomic analysis of β-O-linked glycosylated proteins extracted from glucosamine-treated mouse cumulus-oocyte complexes reveals HSP90 glycosylation as a potential regulator of oocyte competence *abs#021*

1:45pm **Yan Ru Gao**
The role of androgen receptor in mammary gland growth and function in female mice *abs#022*

2:00pm **Stefan Sonderegger**
Interleukin 11 mediates protein secretion and modification in human extravillous trophoblasts - identifying a critical role in placentation *abs#023*

2:15pm **Rasmani Hazra**
Temporal Sertoli cell androgen receptor expression and spermatogenic development in immature testis *abs#024*

2:30pm **Alexander Sobinoff**
Evidence of selective follicular destruction and primordial follicle activation in DMBA induced ovotoxicity *abs#025*

2:45pm **Andrew Reid**
Functional characterisation of the GTPase dynamin in mouse spermatozoa *abs#026*

The SRB acknowledge the support of:

Afternoon Tea

3:00 PM - 3:30 PM Cairns Convention Centre - Upstairs foyer

Embryo development and Implantation

Chairs: Guiying Nie and Georgia Kafer

3:30 PM - 5:00 PM Meeting Room 1+2

3:30pm **Sarah Robertson**
Sperm-borne RNA – a novel role in endometrial receptivity for implantation? *abs#027*

4:00pm **Natalie Hannan**
Endometrial epithelial secreted mediator, VEGF enhances embryo development and implantation *abs#028*

4:15pm **Sarah Paule**
Cleavage of endometrial α-integrins into their functional forms at implantation is mediated by proprotein convertase 6 *abs#029*

4:30pm **Sarah Dalati**
The role of ion channels and membrane potential changes at fertilisation in the mouse oocyte *abs#030*

4:45pm **Gracy Rosario**
Wnt signaling is regulated by leukemia inhibitory factor during mouse embryo implantation *abs#031*

Meat and Livestock Australia and Oozoa Awards

Chairs: Eva Dimitriadis and Chris Grupen

3:30 PM - 5:00 PM Meeting Room 3

3:30pm **Ryan Rose**
Attenuating sheep oocyte nuclear maturation using cAMP modulation *abs#032*

3:45pm **Luis Fernando Malaver-Ortega**
In vitro generation of bovine pluripotent stem cells and their differentiation towards germ cell lineage *abs#033*

4:00pm **Santosh Sahu**
The effect of addition of progesterone (with and without removal of initial gonadotrophin) on oestrus synchronisation in pasture-based dairy heifers *abs#034*

4:15pm **Kara Gunter**
Musashi-2 is responsible for the translational control of *m-numb* in mouse oocytes: a potential role for RNA-binding proteins in meiotic spindle assembly *abs#035*

4:30pm **Nicole Palmer**
Diet and exercise improves sperm function in obese mice *abs#036*

4:45pm **Wai Yuen**
Lack of a Fanconi Anemia DNA repair pathway in oocytes during meiosis may make them susceptible to interstrand crosslinking during prophase I arrest *abs#037*

The SRB acknowledge the support of:

Adelaide Research Centre for Reproductive Health Award Winner

Chair: Kate Loveland

5:00 PM - 5:30 PM Meeting Room 1+2

Caroline Gargett
The endometrium: debut into the stem cell world *abs#038*

The SRB acknowledge the support of:

Student SRB Representative Election and Travel Awards

Chairs: Matt Dun and Chez Viall with SRB Treasurer Rick Nicholson

5:30 PM - 6:00 PM Meeting Room 1+2

Early Career Researcher SRB Representative Election

Chair: Natalie Hannan

5:30 PM - 6:00 PM Meeting Room 3

SRB Awards Dinner

7:00 PM - 11:00 PM Red Ochre Restaurant, Cairns

Sunday, 9 October 2011

Registration Desk Open

7:00 AM - 8:30 AM Cairns Convention Centre - Ground floor foyer

Students and Mentors Breakfast

Chairs: Matt Dun and Chez Viall

7:30 AM - 8:30 AM Meeting Room 3

Invitees: Chris O'Neill, Larry Chamley, Eileen McLaughlin

Growth Factors, Cytokines and Signalling

Chairs: Jim MacFarlane and David Sharkey

8:30 AM - 10:00 AM Meeting Room 1+2

8:30am **Phil Knight**
Intra-ovarian factors regulating thecal androgen production *abs#040*

9:00am **Alison Care**
Macrophage regulation of corpus luteum angiogenesis in early pregnancy *abs#041*

9:15am **Dulama Richani**
Cumulus cell EGF-like peptide and receptor signalling during oocyte in vitro maturation *abs#042*

9:30am **Lisa Akison**
The potential role of the chemotactic signalling axis, CXCR4/CXCL12, during ovulation *abs#043*

9:45am **Peter Mark**
Circadian variation in placental expression of inflammatory mediators *abs#044*

Reproductive and Sexual Development

Chairs: Kaye Stenvers and Brian Setchell

8:30 AM - 10:00 AM Meeting Room 3

8:30am **Hongshi Yu**
The molecular control of reproduction *abs#045*

9:00am **Patrick Western**
Transcriptional and chromatin regulation during male fetal germ cell differentiation *abs#046*

9:15am **Mai Sarraj**
Differential regulation of foetal mouse testis somatic cell markers by TGFβ2 *abs#047*

9:30am **Melissa Gamat**
Morphological and molecular development of the prostate in the tammar wallaby, *Macropus eugenii* *abs#048*

9:45am **Brett Nixon**
Monotremes provide a key to understanding the evolutionary significance of epididymal sperm maturation *abs#049*

Morning Tea

10:00 AM - 10:30 AM Cairns Convention Centre - Upstairs foyer

Lifestyle and Environment: Effects on Development

Chairs: Jock Findlay and Linda Wu

10:30 AM - 12:00 PM — Meeting Room 1+2

10:30am **Paul Fowler**
Environmental effects on fetal reproductive development *abs#050*

11:00am **Tod Fullston**
Diet induced paternal obesity in the absence of diabetes impacts upon developmental and neuromuscular outcomes in neonate offspring in mice *abs#051*

11:15am **Tegan Smith**
13-cis-retinoic acid induces DNA damage in the male germ line *abs#052*

11:30am **Natalie Binder**
Paternal diet-induced obesity retards early embryo development, metabolism, and quality *abs#053*

11:45am **Belinda Nixon**
The consequences of chronic acrylamide exposure on the male germ line *abs#054*

Male Reproduction

10:30 AM - 12:00 PM — Meeting Room 3

Chairs: Lisa O'Donnell and Mark Baker

10:30am **Moira O'Bryan**
Microtubule severing and male fertility *abs#055*

11:00am **Kate Redgrove**
Capacitation-dependent presentation of protein complexes on the surface of human spermatozoa *abs#056*

11:15am **Edward Ottley**
Does activin C promote prostate cancer malignancy? *abs#057*

11:30am **Mark Hedger**
Expression patterns of activin A and its regulatory elements in the adult male mouse reproductive tract indicate important roles for these molecules in the mature epididymis and vas deferens *abs#058*

11:45am **Denise Miles**
Copy number variation in testis cancer *abs#059*

Lunch

12:00 PM - 1:00 PM — Cairns Convention Centre - Upstairs foyer

SRB Council Meeting

12:15 PM - 1:00 PM — Meeting Room 1+2

Early Career Researchers and Mentors Lunch

Chair: Natalie Hannan

12:15 PM - 1:00 PM — Meeting Room 3

Invitees: Phil Knight, Sarah Robertson, Brett Nixon

SRB Presidents Lecture

Chair: Mark Hedger

1:00 PM - 2:00 PM — Meeting Room 1+2

Ray Rodgers
Ovarian extracellular matrix and in health and disease *abs#060*

The SRB acknowledge the support of:

Uterine and Placental Function

Chairs: Lois Salamonsen and Kaushik Maiti

2:00 PM - 3:30 PM Meeting Room 1+2

2:00pm **Mats Brännström**
Uterus transplantation – Animal research towards clinical introduction *abs#061*

2:30pm **Ellen Menkhorst**
Decidual cell surface and secreted factors regulate trophoblast function *abs#062*

2:45pm **Carolyn Mitchell**
Epigenetic mechanisms regulating prostaglandin endoperoxide synthase-2 (PTGS2) expression in the amnion during pregnancy *abs#063*

3:00pm **Jane Girling**
Fibroblast activation markers in ectopic and eutopic endometrium from women with endometriosis *abs#064*

3:15pm **Chez Viall**
Antiphospholipid antibodies and trophoblast shedding: a real phenomenon associated with mitochondrial interactions *abs#065*

Stem Cells and Assisted Reproductive Technologies

Chairs: Bill Kalionis and Huseyin Sumer

2:00 PM - 3:30 PM Meeting Room 3

2:00pm **Justin St John**
Why it is essential to regulate mitochondrial DNA replication during oogenesis and embryogenesis *abs#066*

2:30pm **Jemma Evans**
The fine ‘ART' of endometrial receptivity *abs#067*

2:45pm **John Parrington**
The identification and functional characterisation of mutant isoforms of the oocyte activation factor phospholipase C zeta (PLCζ), and their genetic modes of inheritance *abs#068*

3:00pm **Adam Hart**
Pluripotent stem cells from the testis *abs#069*

3:15pm **Rajneesh Verma**
Generation of Wild Cats iPS cells, using Nanog as an additional factor in the reprogramming cocktail *abs#070*

Afternoon Tea

3:30 PM - 4:00 PM Cairns Convention Centre - Upstairs foyer

SRB Founders Lecture

Chair: Mark Hedger

4:00 PM - 5:00 PM Meeting Room 1+2

Chris O'Neill
On the survival and development of the preimplantation embryo *abs#071*

The SRB acknowledge the support of:

World Congress on Reproductive Biology Welcome Reception

6:00 PM - 7:30 PM Cairns Convention Centre - Upstairs foyer

ABSTRACTS

001

Activin-hormone crosstalk in testis development and dysgenesis: clues from *Smad3* and *Inhibin* mice.

C. Itman[1,2], K. L. Loveland[1,2]

Departments of [1]Biochemistry & Molecular Biology and [2]Anatomy & Developmental Biology, Monash University, Melbourne

Testicular Sertoli cells, the 'nurse cells' to developing sperm, provide the specialized microenvironment essential for spermatogenesis. Appropriate Sertoli cell development and maturation underpin normal adult fertility, hence identifying how these processes are controlled is important for understanding sub/infertility and disorders of testis development. Activin A, a dimeric cytokine formed from inhibin-βA subunits, regulates testis development and fertility by acting locally on testicular cells and distally, promoting pituitary production of follicle stimulating hormone. The closely related inhibin, a heteromeric inhibin-α/inhibin-β dimer, antagonizes activin production and activity. Mice lacking inhibin (*Inha-/-*) develop somatic cell (Sertoli/granulosa) gonadal tumours due to excessive activin signalling. Pituitary gonadotropins, androgens and estrogens exacerbate tumourigenesis in these mice.

To investigate mechanisms underlying dysregulated activin/hormone signalling, we undertook histological and immunohistochemical assessment of *Inha+/+*, *Inha+/-* and *Inha-/-* testes at 7, 16 and 28 days post partum (dpp).

Sertoli cells in dysgenic regions of *Inha-/-* testes exhibited features of immaturity, including mislocalized Connexin43 (blood-testis barrier protein), intense Anti-Mullerian Hormone (AMH) immunoreactivity and irregularly positioned nuclei. Reduced androgen receptor nuclear localization indicated impaired androgen signalling. At 16 dpp, proliferating (PCNA-positive) Sertoli cells were detected and clusters of Sox9-positive cells within the tubule lumen suggested Sertoli cells had detached from the basement membrane. Loss of germ cells was evident at 7 dpp. Unexpectedly, *Inha+/-* mice also had a testicular phenotype, despite being fertile and apparently healthy. Most tubules appeared normal, yet spermatogenesis was advanced, with round spermatids present by 16 dpp. Other tubules contained regions of dysgenesis similar to *Inha-/-* testes, with mislocalized Connexin43 and androgen receptor, increased AMH staining and germ cell loss. In contrast to *Inha* null testes, no PCNA-positive Sertoli cells were detected at 16 dpp and no Sertoli cells were observed in the lumen.

Inha haploinsufficiency therefore results in a mixed testicular phenotype with areas of advanced maturation and areas of impaired development, yet distinct differences exist between dysgenic *Inha+/-* Sertoli cells and tumourigenic *Inha-null* Sertoli cells. Our investigations using *Inha* mice and *Smad3+/-* (advanced testis development) and *Smad3-/-* (delayed Sertoli cell maturation) mice, which have altered activin signalling but do not develop Sertoli cell lesions, are delineating how activin-hormone crosstalk influences testis development and dysgenesis.

002

Kisspeptin and reproduction: examining GnRH response in the median eminence and tracing axon terminals in the ovine brain.

J. Smith

Department of Physiology, Monash University, Melbourne, VIC, Australia

Kisspeptin is the product of the *Kiss1* gene and potently stimulates gonadotropin-releasing hormone (GnRH) secretion. Kisspeptin neurons in the hypothalamus have proven to be an integral part of the 'missing link' bridging the divide between levels of gonadal steroids and feedback control of GnRH secretion. As such, they have proven to be very important in the generation of both estrogen negative and estrogen positive feedback signals to GnRH neurons in females. The former involved in the tonic regulation of GnRH secretion and the latter critical for the rapid and robust rise in GnRH output responsible for the luteinising hormone (LH) surge and ovulation. In sheep, *Kiss1* mRNA expressing cells are found in the arcuate nucleus (ARC) and dorsal preoptic area (POA) and both populations appear to mediate the positive feedback effect of estradiol to generate the preovulatory GnRH/LH surge. In the ovine brain, kisspeptin cells of the POA provide direct input to GnRH neurons, whereas kisspeptin cells of the ARC do not. Data also point to a similar phenomenon in mice. Therefore, alternative pathways from ARC kisspeptin neurons to the GnRH neurons may exist. To demonstrate alternative kisspeptin stimulation of GnRH neurons, we showed kisspeptin effects on GnRH release from ovine median eminence (ME) cultured explants. This indicates direct kisspeptin to GnRH terminal-to-terminal communication within the ME. Using neuronal tracer and immunohistochemical techniques, we examined the origin of kisspeptin neuronal terminals in the external zone of the median eminence. These data suggest ARC populations of kisspeptin neurons send terminals to the median eminence. Overall, our data suggest that kisspeptin has an essential role in receiving estrogen stimulatory signals and generating the full positive feedback GnRH/LH surge. Moreover, we further demonstrate a GnRH-terminal action of kisspeptin and have provided further anatomical data showing the existence and origin of kisspeptin terminals in the ME.

003

Insights into Androgen Action from Tissue-Selective Androgen Receptor Knockout Mouse Models.

U. Simanainen

ANZAC Research Institute, Concord, NSW, Australia

The biological effects of androgens are mediated primarily through the androgen receptor (AR). The recent generation AR knockout (ARKO) mouse models using the Cre/LoxP system has allowed unprecedented selective analysis of androgen actions via AR in target tissues. While male sex accessory glands like prostate and epididymis are well known targets for androgen actions, the role of cell specific androgen actions and the influence of post-testicular androgen actions on male fertility was only recently proven unequivocally by the generation of prostate epithelial AR knockout (PEARKO) mouse model with AR inactivation in prostate and epididymis but not in testes. For the first time we were able to prove that androgen action on the epididymis is essential to complete sperm structural and functional maturation. Despite normal sperm production in the unaffected testes, the kinetics of epididymal sperm passage was impaired in PEARKO males, with increased spontaneous acrosome reaction and abnormal flagellar morphology. The epididymal defect in sperm maturation due to epithelial AR inactivation resulted in progressive and profound subfertility. Unexpectedly, AR inactivation of the prostatic epithelium increased prostate sensitivity to androgens and estrogens and revealed an anti-proliferative role for androgen action via AR in the prostate epithelium. These findings suggest that AR regulates prostate hormonal sensitivity and restrain epithelial proliferation by maintaining mature cell differentiation. Finally, while androgens have a well established role in male sex accessory glands, the androgen insensitive ARKO females uncovered suppressive role for androgens in pubertal mammary growth and susceptibility to experimental breast cancer, demonstrating unsuspected roles of androgen action mediated via AR in the breast. In conclusion, our novel ARKO models have detailed the physiological role of androgens in male and female reproductive tracts and provide an exceptional opportunity for analysis of molecular and cellular mechanisms of sex steroid action and hormonal carcinogenesis.

004

Combination Epidermal Growth Factor Inhibition and methotrexate to medically treat ectopic pregnancies: bench to bedside

S. Tong[1,2,3], U. W. Nilsson[2,3], T. G. Johns[3], T. Wilmann[2], M. Skubisz[1,2], T. Kaitu'u-Lino[1,2], C. Whitehead[1,2], E. Dimitriadis[4], E. Menkhorst[4], B. Saglam[1], Y. Gao[2,3,5], S. Greenall[3], A. Horne[6], E. Wallace[2]

[1]Department of Obstetrics and Gynaecology, University of Melbourne, Heidelberg, VIC, Australia
[2]The Ritchie Centre, Monash Institute of Medical Research, VIC, Australia
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[5]2nd Affiliated Hospital, Xian Jiatong University School of Medicine, China
[6]Centre for Reproductive Biology, The Queen's Medical Research Institute, Edinburgh, United Kingdom

Ectopic pregnancies occur when the conceptus implants outside the uterus, predominantly in the Fallopian Tube. They can rupture through the ectopic site causing fatal intra-abdominal bleeding. While methotrexate efficaciously regresses small ectopic pregnancies, most (75%) are too large and require surgical excision. Since placenta has the highest expression of Epidermal Growth Factor Receptor (EGFR) expression of all tissues, its inhibition could be a novel way to treat ectopic pregnancy. We investigated whether combining gefitinib (orally available EGFR inhibitor) with methotrexate could be a novel approach to efficaciously resolve ectopic pregnancies. Gefitinib and methotrexate was supra-additive in inducing inhibition or death *in vitro* when co-administered to placental cells representing different layers of the first trimester placenta. Mechanistically, gefitinib blocked pro-survival Akt activation induced by methotrexate. Conversely, methotrexate enhanced gefitinib's ability to block EGFR activation. Combining the agents increased apoptosis compared to either drug alone. Gefitinib or methotrexate alone induced dose-dependent decreases in the size of placental cell xenografts implanted subcutaneously in SCID mice, but combining them was supra-additive. Compared to either agent alone, co-administration of these agents doubled rates of fetal resorption in immuno-competent mice. Co-administration of methotrexate and gefitinib to nine women diagnosed with ectopic pregnancy was well tolerated and induced a more rapid decline in serum human chorionic gonadatrophin compared to controls treated with single agent methotrexate. We conclude combining EGFR inhibition with methotrexate may represent a novel non-surgical approach to treat unruptured ectopic pregnancies of any size.

005

The role of histone variant h2afz in trophoblast differentiation: implications for the development of *in vitro* generated preimplantation mouse embryos.

G. R. Kafer[1], D. G. Simmons[1], M. Pantaleon[1], P. L. Kaye[1], S. A. Lehnert[2]

[1]School of Biomedical Sciences, The University of Queensland, St Lucia, QLD, Australia
[2]Livestock Industries, CSIRO, St Lucia, QLD, Australia

The dynamic incorporation and expulsion of histone variants from chromatin along with the activity of chromatin remodelers can establish transcriptionally permissive and repressive chromatin environments. The histone variant H2AFZ is thought to act as a master controller of chromatin mediated transcriptional regulation and assist in mediating cell plasticity due to its documented localisation at both transcriptional start sites and in heterochromatin (*1, 2*). In these studies, we found that the histone variant *H2afz* was highly expressed in *in vivo* mouse blastocysts relative to other preimplantation development stages (*3*) ($P<0.05$, $n\geq3$, mRNA from 3 embryos assayed/experiment/stage, exogenously normalized (*4*)) and that *H2afz* expression was significantly lower in *in vitro* blastocysts (KSOM cultured 16 – 92 h post-hCG, $P<0.05$, $n\geq3$, mRNA from 3 embryos assayed/experiment/stage/type, endogenously normalised to *Rplp0*), a finding supported by western immunoblotting experiments using an antibody specific for H2AFZ (5) ($n\geq3$). In mouse blastocysts H2AFZ is only present in the trophectoderm (*6*). We aimed to investigate the expression and localisation of *H2afz* in the developing mouse placenta, hypothesising that *H2afz* is limited to uncommitted trophoblast cells. qRT-PCR revealed *H2afz* was present in undifferentiated trophoblast stem cells and that levels declined following differentiation ($P<0.05$, $n\geq3$, endogenously normalised to *Rplp0*). Western immunoblotting confirmed these results ($n\geq3$). *In situ* hybridisations using DIG-labelled *H2afz* riboprobes on mouse placental sections (embryonic day (E)6.5 - E18.5) showed that *H2afz* was widely expressed in proliferating, progenitor cell populations within the ectoplacental cone and chorion (E7.5 - E10.5). As mouse placentation continued, *H2afz* became restricted to the labyrinth in small clusters of cells that did not exhibit morphological characteristics of committed trophoblast cells (E14.5 - E18.5) ($n\geq3$). These data raise important questions regarding the susceptibility of *H2afz* to different developmental milieu and the potential impacts of abnormal H2AFZ on extra-embryonic tissue differentiation, implantation and embryo development.

(1) Creyghton MP, Markoulaki S, Levine SS, Hanna J, Lodato MA, Young RA, Jaenish R, Boyer LA (2008). Cell, 135(4).
(2) Hardy S, Jacques PE, Gevry N, Forest A, Fortin ME, Laflamme L, Gaudreau L, Robert F (2009). PLoS Genetics, 5(10).
(3) Kafer GR, Lehnert SA, Pantaleon M, Kaye PL, Moser RJ (2010). Gene Expression Patterns, 10(6).
(4) Bower NI, Moser RJ, Hill JR, Lehnert SA (2007). BioTechniques, 42(2).
(5) Kafer GR, Moser RJ, Pantaleon M, Kaye PL, Lehnert SA. Cellular Reprogramming, In Press (June, 2011).
(6) Rangasamy D, Berven L, Ridgway P, Tremethick DJ (2003). EMBO Journal, 22(7).

006

Identification of the Renin angiotensin system (RAS) pathways in BeWo and HTR-8/SVneo cells

Y. Wang, K. G. Pringle, T. Zakar, E. R. Lumbers

Mothers and Babies Research Centre, Hunter Medical Research Institute, John Hunter Hospital, University of Newcastle, Newcastle, NSW, Australia

The renin-angiotensin system (RAS) regulates trophoblast invasion, which is critical for placental development (1). Impaired placental development contributes to preeclampsia and intrauterine growth restriction (2&3). To characterise the RAS pathways in two trophoblast cell lines (BeWo and HTR-8/SVneo), we measured prorenin (*REN*), prorenin receptor (*ATP6AP2*), angiotensinogen (*AGT*), angiotensin II type 1 and 2 receptor (*AGTR1* and *AGTR2*), angiotensin converting enzyme 1 and 2 (*ACE1* and *ACE2*), angiotensin (Ang) 1-7 receptor (*MAS1*) and promyelocytic zinc finger protein (*PLZF*) mRNA levels using qRT-PCR. Prorenin protein, Ang II and Ang 1-7 peptides were measured in the incubation medium using ELISA and RIA respectively.

After 24 and 48h incubation, both BeWo and HTR-8/SVneo cells expressed *AGT*, *ATP6AP2* and *ACE1*. HTR-8/SVneo cells had significantly lower *AGT* and higher *ATP6AP2* expression than BeWo cells (both $P<0.001$). Only HTR-8/SVneo cells expressed *REN* and *AGTR1* and their abundance increased with incubation ($P=0.019$ and $P=0.001$, respectively), whereas *ACE2, MAS1* and *PLZF* were only expressed in BeWo cells. *AGTR2* mRNA was not expressed in either cell line.

Prorenin was found only in media from HTR-8/SVneo cells (0.31 Â± 0.06 ng/mL), while Ang II and Ang 1-7 peptides were synthesised by both BeWo and HTR-8/SVneo cells. Despite the absence of detectable expression of genes required for their generation, both Ang peptides were produced, suggesting that other Ang forming proteases are present in these cell cultures.

In conclusion, HTR-8/SVneo cells express genes of the Ang II/AT_1R pathway and the prorenin/prorenin receptor pathways. This is very similar to the pattern of RAS gene expression seen in early gestation placenta (4), therefore this cell line is suitable for studying the RAS in early gestation. BeWo cells only express genes for the Ang 1-7/Mas receptor pathway and may be useful for studying the actions of Ang 1-7.

(1) Xia Y, Wen HY, Kellems RE 2002 Angiotensin II inhibits human trophoblast invasion through AT1 receptor activation. J Biol Chem 277:24601-24608

(2) Arroyo JA, Winn VD 2008 Vasculogenesis and angiogenesis in the IUGR placenta. Semin perinat 32:172-177

(3) Bdolah Y, Sukhatme VP, Karumanchi SA 2004 Angiogenic imbalance in the pathophysiology of preeclampsia: newer insights. Semin nephrol.

(4) Pringle KG, Lumbers ER Gestational changes in the expression of the placental renin-angiotensin system and VEGF: roles in placental vascularisation? Fetal and neonatal physiology workshop. Hobart, Apr

007

The Effects of Dietary Omega-3 Fatty Acids on Inflammatory and Oxidative Status of the Rat Placenta

M. L. Jones, P. J. Mark, B. J. Waddell

School of Anatomy & Human Biology, The University of Western Australia, Nedlands, WA, Australia

Placental inflammation and oxidative stress play key roles in the pathophysiology of placental-related disorders. Protection from oxidative stress is provided by antioxidant enzymes which inactivate reactive oxygen species. Omega-3 (n3) polyunsaturated fatty acids (PUFAs) are proposed to have both anti-inflammatory and antioxidant properties, potentially by up-regulating antioxidant enzyme expression. Here, we tested the hypothesis that dietary n3-PUFA intake reduces the inflammatory and oxidative status of the placenta.

Pregnant rats consumed a high n3-PUFA (Hn3) or control diet from day 1 of pregnancy. Fetuses and placentas were collected on days 17 or 22 (term=23), and placentas dissected into junctional (JZ) and labyrinth (LZ) zones. Placental gene expression of pro-inflammatory mediators TNFα, IL-6, IL-1β, COX1 and COX2, and of antioxidant enzymes catalase and SOD2 were measured by qRT-PCR (males only).

Hn3 consumption increased fetal ($P<0.05$) and placental ($P=0.05$) weights at gestational day 22 (6.2% and 10.6% respectively). Overall, LZ and JZ expression of all genes increased ($P<0.05$) from day 17 to 22. At day 17, Hn3 intake decreased IL-6 expression in both zones ($P<0.05$) and IL-1β in JZ ($P<0.05$), but by day 22 these effects were no longer evident. On the contrary, LZ expression of IL-6 (2.7-fold, $P<0.05$) and IL-1β (1.7-fold, $P<0.05$) at day 22 were both increased by the Hn3 diet. Dietary Hn3 increased placental expression of catalase in the LZ at both days ($P<0.001$), but decreased both catalase and SOD2 in JZ at day 17 ($P<0.05$ and $P<0.001$ respectively).

In conclusion, dietary n3-PUFA supplementation increased fetal and placental growth, which was associated with a reduction in pro-inflammatory cytokine gene expression at gestational day 17. These anti-inflammatory effects were no longer apparent by day 22, possibly due to the rapid increase in cytokines approaching parturition. Dietary n3-PUFAs also affected antioxidant expression in a zone-specific manner.

008

Low oxygen and cell-extracellular matrix interactions alter expression of invasion-modulating genes in the first trimester trophoblast cell line, HTR8/SVneo.

A. R. Highet, C. T. Roberts

Research Centre for Reproductive Health, Robinson Institute, University of Adelaide, Adelaide, SA, Australia

Background: Invasion of extravillous cytotrophoblast cells (EVCT) into the maternal decidua is critical to placentation, with insufficient invasion associated with pregnancy complications. EVCTs adhere to the decidual extracellular matrix (ECM) by integrin binding. This interaction transduces signals which affect cellular behaviour, such as expression of matrix metalloproteinases (MMPs) that facilitate ECM degradation and TGF-β which restrict invasion. The early first trimester placenta is relatively avascular and the hypoxic environment is thought to promote invasion. We hypothesised that low oxygen and ECM in culture up-regulate expression of genes that promote ECM degradation and invasion by trophoblasts.

Methods: HTR8/SVneo were cultured for 24h on 15μM growth factor reduced (GFR) Matrigel, 1:2 diluted GFR Matrigel or uncoated plastic, in 1%, 5% or 20% O_2. Expression of integrin genes *(ITG) A1, A5* and *B1, TGFB1, TGFB2, MMP2* and *MMP9* were quantified by qPCR. Normalised relative expression data were analysed using ANCOVA and pairwise t-tests.

Results: Cells cultured on plastic had significantly higher expression of *ITGA1, ITGA5, TGFB1, MMP2* and *MMP9*. Expression of *ITGB1* and *TGFB2* were not affected by culture surface. After correction for Matrigel, low oxygen significantly increased expression of all genes except MMP2 ($p<0.05$).

Conclusions: Low oxygen and ECM interactions alter expression of invasion-modulating genes in HTR8/SVneo *in vitro*, but expression of *ITG, TGFB* and *MMP* mRNA is either lower on ECM than on plastic, or in the case of *ITGB1* and *TGFB2* not affected. Plastic culture surface does not accurately represent the *in vivo* environment of trophoblast cells and could induce aberrant gene expression. Increased expression of *MMP9* and *ITGA1 and ITGA5* in low oxygen supports the contention that a hypoxic environment promotes invasion.

009

Effect of dietary vitamin D and calcium deficiency on pregnancy status in C57Bl6 female mice

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Intro: Vitamin D (vitD) and calcium (Ca) deficiencies have been associated with a range of pregnancy complications including preeclampsia, small-for-gestational age babies and preterm labour in humans and a 75% decrease in fertility in rats. VitD is known to maintain Ca homeostasis through upregulation of intestinal Ca absorption. In addition, the placenta is a major site of vitD synthesis and activity. We aim to determine the relative contribution of dietary vitD and Ca on pregnancy outcomes and placental physiology in a mouse model.

Methods: Female C57BL6 mice were fed one of 8 assigned diets from 3 weeks old. The control diet contained a standard 1000 IU vitD + 1% Ca, while deficient diets were modified for Ca (1% or 0.1%) and vitD (1000, 500, 100 or 0 IU/kg vitD). At 12 weeks of age female mice were mated, then killed at day 18.5 post-coitum for fetal and placental analyses.

Results: Preterm births were observed on day 18 of gestation (term = day 19-20) in mice exclusively from deficient diets, specifically control and 1000 IU vitD + 0.1% vs 0 IU VD + 0.1% Ca ($P<0.05$). Litter size, fetal and placental weights were not different, however maternal body composition was altered, with pregnant mice having significantly different lung weights ($P=0.025$), while non-pregnant mice differed by liver and parametrial fat weights ($P=0.022$, $P=0.031$) between diets.

Conclusion: This study demonstrates that diets deficient in vitD and Ca are associated with increased incidence of preterm birth. Placental morphology and gene expression with respect to serum vitD and Ca are yet to be undertaken. Further analysis will elucidate the roles of dietary vitamin D and calcium in placental physiology and pregnancy.

010

Plasma prorenin levels at 15 weeks gestation in women who had normal and complicated pregnancies

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Prorenin is a precursor to active renin. The renin angiotensin system regulates blood pressure and fluid and electrolyte balance. To determine if maternal plasma prorenin concentration in early gestation is an indicator of pregnancy outcome, a subset of women participating in the Adelaide SCOPE (screening for pregnancy endpoints) cohort were assessed according to pregnancy outcome as either normal (n=131) or pathological, having either gestational diabetes (GD, n=36), gestational hypertension (GHT, n=50), preeclampsia (PE, n=50), preterm birth (PTB, n=50) or small for gestational age babies (SGA, n=50). Maternal blood was collected at 15 weeks gestation and prorenin measured using an ELISA (Molecular Innovations, MI, USA). Plasma prorenin concentration was not different between women with a normal compared to complicated pregnancy outcome (Table 1). There was no difference in maternal plasma prorenin concentration in women carrying male versus female fetuses. However in women destined to develop GHT, plasma prorenin at 15 weeks gestation was lower in those carrying a female versus a male fetus (2.40 ± 0.19 ng/mL, n=21 vs. 3.20 ± 0.23 ng/mL, n=29, P=0.019). These data suggest that fetal sex may influence maternal plasma prorenin concentration in GHT. Since the kidney is likely the major source of maternal plasma prorenin, renal prorenin secretion may be regulated differently according to fetal sex in those pregnancies destined to develop hypertension. Pregnancies that are destined to develop PE did not show this sexual dimorphism in prorenin levels, a finding which remains to be explained.

Table 1: Pregnancy pathologies of the Adelaide SCOPE cohort and maternal prorenin levels at 15 weeks gestation

Pregnancy Pathology	Maternal Prorenin (ng/mL)
Normal Pregnancy	3.07 ± 0.13
Gestational Diabetes	3.41 ± 0.29
Gestational Hypertension	2.86 ± 0.16
Preeclampsia	3.36 ± 0.25
Preterm Birth	3.33 ± 0.22
Small for Gestational Age	3.01 ± 0.21

011

Oocyte maturation and ovulation, coordination by the cumulus complex.

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Ovarian function involves continuous cycles of follicle growth and ovulation or regression controlled by endocrine and local growth factor signals. Infertility and polycystic ovary syndrome are both increasingly common conditions associated with dysregulated ovarian tissue remodelling. We aim to reveal the molecular mechanisms of controlled tissue morphogenesis in ovaries, and determine how the changing ovarian follicle environment promotes the growth and release of healthy oocytes. Our recent work elucidates key mechanistic actions of the cumulus oocyte complex (COC) matrix coordinating maturation and ovulation of oocytes. We have demonstrated that COC matrix genes are required for ovulation as well as causally linked with oocyte and subsequent embryo developmental competence. The COC may mechanistically mediate ovulation through transiently induced invasive migration of cumulus cells at the time of ovulation. Specifically, intact COC from mice stimulated to initiate ovulation acquire the capacity to migrate towards a chemotactic stimulus, with motility peaking 12 h after hCG treatment (the time of ovulation) then rapidly downregulated. In the same time frame COC acquire the capacity to adhere to collagens and to invade a 3-dimensional collagen type-I matrix barrier similar in composition to the ovarian wall which must be breached during ovulation. Overall these studies indicate that invasive migration of COCs, controlled by endocrine and oocyte-derived signals impinging on cumulus cells, is a key aspect of successful release of oocytes from the ovary. To support and coordinate oocyte maturation and ovulation the COC matrix exerts context dependent signalling actions through regulated expression of heparan sulphate proteoglycans that control interactions of growth factors with their receptors on cumulus cells. Thus, formation of the uniquely specialised COC matrix is a critical nexus in maturation of high competence oocytes and their ovulation from the ovary.

012

Sub-fertility in mice lacking functional androgen receptors in granulosa cells

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Paracrine interactions between the oocyte and surrounding granulosa cells are critical for normal ovarian follicle development. Within the ovary, granulosa cells display strong androgen receptor (AR) expression and are exposed to the potent endogenous AR agonists, testosterone and dihydrotestosterone, so that granulosa cells may constitute a major site of AR-mediated action in the follicle. Using Cre/LoxP recombination we generated granulosa cell specific androgen receptor (AR) knockout mice (GCARKO) by mating transgenic AMH-Cre mice with Arflox mice (exon 3 flanked by loxP sites). This selectively deletes the AR second zinc finger essential for DNA-binding and creating a minimally truncated but non-functional AR protein in granulosa cells. Resulting GCARKO females were sub-fertile producing fewer litter (GCARKO: 5.4 +/- 0.6; WT: 7 +/- 0,

P<0.05), and an age-dependent reduction in cumulative pups per month, evident from 4 months of age (P < 0.05), compared with WT female breeders. This resulted in GCARKO female breeders taking longer to produce 30 pups (median GCARKO = 129 days, WT 83 days, P<0.05). Between genotypes there was no difference in estrous cycling (average cycle length in days GCARKO: 5.7 +/- 0.6; WT: 4.9 +/- 0.4), body, ovary or uterus weights at 12, 26 or 52 weeks of age, or hormone levels at 12 weeks of age. At 12 weeks of age, GCARKO had a marginally significant reduction in small antral follicle numbers (P=0.05). There was no difference between other growing follicle populations or corpus lutea counts. There was also no difference in ovarian gene expression levels of Fshr, Igf1r or KitL between genotypes at 12 weeks of age. In conclusion, granulosa cell AR action is a site of requirement for normal female fertility. However, the dysfunctional mechanisms leading to the observed age-related sub-fertility require further elucidation.

013

FoxL2 is involved in GDF-9 and activin regulation of follistatin in granulosa cells: GCT FoxL2^{C134W} mutation exerts dominant activity.

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FoxL2 is a member of the forkhead transcription factor family required for normal ovary and eyelid development. A single somatic *FOXL2* mutation (*FOXL2*^{C134W}) has been identified in over 90% of adult granulosa cell tumors (GCTs). Cooperative regulation of activin signaling by FoxL2 and Smad3 has been established in the pituitary, thus we predicted a similar FoxL2 involvement in the Smad3-signaling of GC mitogens, activin and GDF-9, would occur in the ovary. One pituitary target of FoxL2/Smad3 cooperative actions is follistatin, which is highly expressed in GCs of larger follicles and negates activity of several ovarian mitogens, including activins, BMP-7 and BMP-15. Thus, dysregulation of follistatin by FoxL2^{C134W} has the potential to significantly alter GC proliferation. We found that GDF-9 and activin both stimulate follistatin mRNA expression, and that this regulation is at the level of transcription and requires the Smad3/FoxL2 binding element located in the first intron. Unexpectedly, we found that FoxL2 has an *opposite* role in regulating GDF-9- and activin-driven follistatin expression in primary rat GCs to that observed in pituitary cells. Specifically, siRNA knockdown of endogenous FoxL2 increased activin and Smad3 stimulation of follistatin luciferase activity, whereas over-expression of FoxL2 suppressed GDF-9 and activin stimulation of the follistatin promoter, supporting an inhibitory role for FoxL2 in follistatin regulation. Moreover, over-expression of the FoxL2^{C134W} mutant suppressed activin activity more than the wt FoxL2, suggesting that FoxL2^{C134W} acts as a gain-of-function mutation in primary rat GCs in terms of follistatin transcriptional regulation. An inhibitory role for FoxL2^{C134W} in regulating follistatin is supported by follistatin immunostaining using human ovaries that was strongly positive in GCs of large antral follicles and low in GCTs. The consequential increase in availability of GC mitogens due to enhanced suppression of follistatin expression may support the malignant growth of GCs in GCT patients.

014

Loss of pro-apoptotic proteins, Puma and Noxa, prevents oocyte death and preserves fertility following anti-cancer therapy-induced DNA damage.

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A common and serious side effect of DNA damaging anti-cancer therapy is death of primordial follicle oocytes, resulting in premature ovarian failure and female sterility. Trp63, a Trp53 homologue, is expressed in the nucleus of primordial follicle oocytes and is essential for their death following DNA damage. However, the mechanisms by which Trp63 mediates oocyte death through apoptosis under physiological conditions are unknown. Using gene-targeted mice, we show that the pro-apoptotic proteins Puma and Noxa are critical down-stream apoptosis effectors for Trp63 following DNA damage in primordial follicle oocytes. Within 3 hours of Î³-irradiation, *Puma* and *Noxa* were transcriptionally induced in oocytes of wild type and *Trp53*$^{-/-}$ mice but not in those lacking Trp63. Furthermore, Î³-irradiation resulted in loss of all primordial follicles in wild type and *Trp53*$^{-/-}$ mice within 5 days of treatment. By contrast, a cohort of primordial follicles were protected from apoptosis in mice lacking Puma (16% Â± 3%), and even more profoundly in mice lacking both Puma and Noxa (52% Â± 6%, p<0.001 vs. wt). Remarkably, the quality of rescued oocytes was confirmed by the generation of healthy offspring from Î³-irradiated *Puma*$^{-/-}$ and *Puma*$^{-/-}$*Noxa*$^{-/-}$ mice. These data suggest that Trp63-mediated apoptosis in primordial follicle oocytes may be inhibited through blockade of Puma in order to preserve female fertility during anti-cancer therapy.

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015

The APC/C activator FZR1 controls the timing of oocyte meiotic resumption in mammalian oocytes in a phosphorylation-dependent manner

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The Anaphase-Promoting Complex/Cyclosome (APC/C) plays an essential role in co-ordinating meiotic maturation of the mammalian oocyte in concert with two temporally distinct co-activator proteins. Both co-activators, FZR and FZR1 have well established roles during the mitotic cell cycle but the meiotic role of FZR1 remains unclear. In order to examine the role of this protein in the mature mouse oocyte, we generated an oocyte-specific Cre/lox knockout of the FZR1 gene using Zp3 promoter-driven Cre recombinase activity. Oocytes must normally remain Prophase I/Germinal Vesicle (GV) arrested until ovulation, however the ability of FZR1Δ/Δ oocytes to remain GV arrested was compromised both in vivo and in vitro. Protein levels of the APC/CFZR1 substrate Cyclin B1 were elevated >5-fold in FZR1Δ/Δ oocytes. Precocious meiotic entry was attributed to cyclin B1-induced activation of CDK1 activity. Rescue of APC/C FZR1 activity and therefore the normal timing of GVB could be achieved by microinjecting

FZR1 copy-RNA into FZR1Δ/Δ oocytes in vitro. Since phosphorylation has been found to play a role in regulating FZR1 localization and activity in somatic cells we also tested the ability FZR1 phosphorylation-site mutants to localize to the nucleus and restore APC/CFZR1 activity in FZR1 Δ/Δ oocytes. Hypo-phosphorylation mutants in which up to 9 phosphorylation sites were mutated to alanine were able to increase time until GVB beyond that seen for wild-type FZR1, however hyper-phosphorylated mutants failed to rescue the normal timing of GVB and APC/CFZR1 activity. Phosphorylation of these sites also decreased FZR1 nuclear accumulation relative to the cytoplasm. Thus FZR1 plays an important role in preventing precocious GVB in the mouse through maintaining degradation of cyclin B1, and its activity and subcellular localization are regulated in a phosphorylation-dependent manner.

016

Mammalian meiosis: cell cycle regulation and male fertility

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Accurate chromosome segregation during mitosis and meiosis is facilitated by a regulatory complex known as the Anaphase Promoting Cyclosome (APC), a ubiquitin ligase complex that tags proteins that are subsequently recognised by the 26S proteosome and degraded. In mammalian cells, two temporally regulated co-activators are required for the APC function; fizzy (fzr) and fizzy related 1 (fzr1). While studies of oocyte development have demonstrated fzr1 plays an important role in maintaining G2 arrest during meiosis by controlling spatial levels of the cell cycle protein Cyclin B1, the role of fzr1 in spermatogenesis has remained uncharacterised. Our preliminary investigations indicated that fzr1 is expressed in the developing male germ cell and that gene and protein expression levels are elevated in both meiotic and post meiotic gem cells, suggesting fzr1 plays an important role in spermatogenesis. Germ cell specific conditional knockout *fzr1*mice were generated using DDX4-Cre and floxed fzr1 mouse lines. Adult *fzr1* null mice are sterile and exhibit spermatogenic arrest with complete absence of round spermatids and concomitant apoptosis in the residual spermatocytes. Markers of spermatogenic cell cycle indicated that germ cell mitotic development was normal in young adult null males. Germ cell arrest was localised to early meiotic development and meiotic spreads probed confirmed that null germ cells largely arrested in late zygonema and only rarely were early pachytene spermatocytes identified. This unexpected finding led to the investigation of a number of the known targets of APC^{fzr1} for proteosomal degradation. Using immunoprecipitation and proximity ligation assays, we confirmed that in addition to known targets the transcription factor, Ets2 and Cyclin B1 and a novel target protein is significantly upregulated in null germ cells. From these findings we hypothesize that APC^{fzr1} mediated destruction of Ets2, Cyclin B1 and other novel targets are essential mediators of meiotic progression and meiotic division in male spermatogenesis.

017

The Critical Requirement for Wnt signalling in spermatogenesis

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Male infertility is a worldwide problem with increasing incidence that may be caused in part by disruptions in testicular cell communication between male germ cells and their supporting somatic cells. The Wnt signalling pathway is highly conserved throughout evolution and plays an essential role in diverse processes including maintenance of stem cells, cellular proliferation and differentiation. We have recently identified an essential role for regulation of Wnt signalling during spermatogenesis via the use of genetically altered mice with perturbed Wnt signalling that exhibit interrupted spermatogenesis and reduced fertility. While a key role for Wnt signalling has been identified in Sertoli cell differentiation,our data revealed a role for canonical Wnt signalling in post-mitotic germ cell development. We are currently using two unique mouse models to understand the precise function of Wnt signalling during spermatogenesis. These allow us to observe the effect of blocking Wnt signalling by acute mutation of beta-catenin, a key mediator of canonical Wnt signalling. In contrast, the effects of constitutively active Wnt signalling is being studied following conditional mutation of the negative regulator, Adenomatous Polyposis Coli (APC).

018

The Regulation of Sertoli Cell Micro-RNAs by TGFβ3

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Most events of spermatogenesis, including meiosis, spermiogenesis and spermiation, occur within a unique adluminal microenvironment created by the blood–testis barrier (BTB), which is in part comprised of specialised junctions between Sertoli cells. However, the molecular mechanism(s) by which BTB restructuring occurs are poorly understood. Members of the TGFβ superfamily, including TGFβ2, TGFβ3 and GDF9, are present at stage VIII and can disrupt the BTB. In other epithelial cell types (breast, kidney), TGFβ-induced cell junction restructuring is mediated, in part, via micro-RNAs (miRNAs), which are small non-coding RNAs that post-transcriptionally regulate gene expression. We therefore hypothesised that TGFβ3 regulates Sertoli cell miRNAs which target junction-associated proteins important in the BTB. We aimed to identify TGFβ3-regulated miRNAs in rat Sertoli cells *in vitro*, and to correlate these with potential junctional targets. Addition of TGFβ3 to rat Sertoli cells *in vitro* caused time- dependent (1-10hr) decreases in Sertoli cell tight junction function. Comparative microarray analysis was then used to characterise TGFβ3-specific miRNAs produced by rat Sertoli cells. Of a total of 353 miRNAs commonly expressed in both TGFβ3- and control treated cells, 13 were significantly up-regulated by TGFβ3 and 12 miRNAs were down-regulated. Bioinformatic prediction of miRNA-mediated pathways using the combined up-regulated miRNAs revealed adherens junctions, focal adhesions, and the actin cytoskeleton, to be amongst the four top pathways represented. These pathways have known roles in the specialised junctions between Sertoli cells which contribute to the BTB, suggesting that TGFβ-mediated miRNAs may impact on their function. This is supported by recent evidence from our lab which demonstrated that hormonally-mediated miRNAs in Sertoli cells target cell adhesion pathways. We conclude that TGFβ3 mediates a discrete subset of Sertoli cell miRNAs which potentially impact the regulation of Sertoli cell junctions important in the function of the BTB.

019

Paraspeckle protein 1 nuclear import is mediated by importin alpha 2; implications for testicular paraspeckle function

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Spermatogenesis is dependent on sequential, tightly controlled changes in gene expression, coordinated by a plethora of transcription and other nuclear factors. Importin proteins are central to these changes through facilitating nuclear transport dependent on targeting signals such as nuclear localisation sequences (NLSs). Importin expression is regulated throughout spermatogenesis [1], with mouse importin alpha 2 (KPNA2) transcript levels peaking at E12.5 in the embryonic testis and within spermatocytes and spermatids of the adult testis. Importantly, a yeast 2-hybrid screen of an E12.5 mouse testis cDNA library identified paraspeckle protein 1 (PSPC1) as an importin alpha 2 interactor. PSPC1 is a defining marker of paraspeckles, distinct subnuclear structures formed around the non-coding RNA transcript, NEAT1 [2], in which A-to-I edited RNA transcripts are believed to be retained in the nucleus. The long PSPC1 isoform is highly expressed in testis [3], and thought to regulate androgen receptor-mediated transcriptional activity [4]. Since PSPC1's functions are exclusively nuclear, we hypothesised that PSPC1 trafficking into the nucleus and subsequent paraspeckle localisation may be mediated specifically by importin alpha 2. We firstly used recombinant proteins and ELISA based binding assay to confirm direct, high affinity interaction between PSPC1 and importin alpha 2 as well as importin alpha 6 but not alpha 4. That this was a functional interaction in terms of nuclear transport was confirmed by quantitative confocal laser scanning microscopy demonstrating enhancement of nuclear import by full length importin alpha 2 in HeLa cells, and inhibition thereof by a truncated (dominant negative) derivative of importin alpha 2. Importantly, inhibition of PSPC1 nuclear import by dominant negative importin alpha 2 reduced the number of detectable paraspeckles. The clear implication is that the alteration of specific importin levels during spermatogenesis is likely to affect cell function, including through controlling the assembly of RNA processing machinery.

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020

Phosphoinositide-3-kinase signaling pathway involvement in a truncated apoptotic cascade associated with motility loss and oxidative DNA damage in human spermatozoa

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Human spermatozoa are characterized by poor functionality and abundant DNA damage that collude to generate the high incidences of male infertility and miscarriage seen in our species. Apoptosis is a highly-characterized process of cell death with well known roles in embryogenesis and development. Recent evidence has highlighted significant relationships between apoptotic markers in human spermatozoa and decreased sperm function. However, the ability of spermatozoa to enter an apoptotic state and the factors that might trigger such an event are unresolved. Consequently, the aim of our study was to for the first time characterize elements of the apoptotic pathway in human spermatozoa. In this study we present evidence that the commitment of these cells to apoptosis is negatively regulated by PI3 kinase/AKT. If PI3 kinase activity is inhibited then spermatozoa default to an apoptotic cascade characterized by rapid motility loss, mitochondrial reactive oxygen species generation, caspase activation in the cytosol, annexin V binding to the cell surface, cytoplasmic vacuolization and oxidative DNA damage. However, the specialized physical architecture of spermatozoa subsequently prevents endonucleases activated during this process from penetrating the sperm nucleus and cleaving the DNA. As a result, DNA fragmentation does not occur as a direct result of apoptosis in spermatozoa as it does in somatic cells, even though oxidative DNA adducts can clearly be detected. We propose that this unusual truncated apoptotic cascade prepares spermatozoa for silent phagocytosis within the female tract and prevents DNA-damaged spermatozoa from participating in fertilization. In light of these findings, we would expect that growth factors/cytokines that stimulate PI3 kinase/AKT would have a powerful pro-survival effect on human spermatozoa.

021

Proteomic analysis of β-O-linked glycosylated proteins extracted from glucosamine-treated mouse cumulus-oocyte complexes reveals HSP90 glycosylation as a potential regulator of oocyte competence.

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Glucosamine is a well characterised hyperglycaemic mimetic, and supplementation during in vitro maturation (IVM) decreases mouse oocyte developmental competence through increased O-linked glycosylation [1]. O-linked glycosylation of proteins within cumulus cells occurs as a result of increased hexosamine biosynthesis pathway (HBP) activity, the pathway that provides substrates for matrix production during maturation, but also for O-linked glycosylation. Here, we sought to identify target proteins that are O-linked glycosylated within cumulus oocyte complexes (COCs) in the presence of glucosamine.

Mouse COCs were incubated for 18h +/- 2.5mM glucosamine +100mIU/ml FSH. Immunoprecipitation of COCs with an anti-β-O-linked glycosylation antibody (CTD110.6) revealed that the addition of glucosamine increased detectable O-glycosylated protein levels and that this was reversed in the presence of an O-linked N-acetylglucosamine transferase (OGT) inhibitor (benzyl-2-acetamido-2-deoxy-α-D-galactopyranoside, 2.5mM). This was seen throughout maturation (0, 6, 12 and 18h). The immunoprecipitated product was separated by gel electrophoresis and excised bands analysed using liquid chromatography-electrospray ionisation ion-trap mass spectrometry to identify potential glycosylated protein targets. Several candidate proteins were identified, including HSP90α and HSP90β. Inhibition of HSP90 significantly reduces the expression and half life of OGT in bovine aortic endothelial cells, and reduces O-glycosylation levels [2], suggesting this enzyme is a client protein of HSP90. We tested the effect of inhibiting HSP90 within COCs treated with glucosamine during IVM using 0.1μM 17-(allylamino)-17-demethoxygeldanamycin. This HSP90 inhibitor was able to reverse the glucosamine-induced decrease in oocyte competence, suggesting an aberrant function of glycosylated HSP90.

This project is the first to identify potential candidate proteins that are aberrantly O-glycosylated under hyperglycaemic conditions during COC maturation. The identification of these protein targets is an important step in elucidating the mechanism by which hyperglycaemia leads to reduced fertility, especially during the periconception period.

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022

The role of androgen receptor in mammary gland growth and function in female mice

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The Androgen receptor (AR) is expressed and testosterone produced in situ in normal mammary cells of women and female mice, indicating a role for AR in mammary growth and function. To gain insight into AR functions in the mammary gland, we used AR knockout (ARKO) mice that have a global in-frame deletion of exon 3 of the AR gene (Cre-LoxP) leading to the production of a transcriptionally inactive AR protein. At puberty (5 weeks of age), ARKO mammary glands displayed accelerated epithelial growth determined by whole mount staining. The ductal distance was greater in ARKO than in WT mammary glands (2.9±0.6 [mean±SD] vs 1.3±0.5mm, p=0.025) and the ARKO female had more terminal end buds compared to WT (18.6±2.1 vs 7.2±2.5, p=0.021). Yet, the serum progesterone and intra-ovarian estradiol were comparable. In contrast, virgin, sexually mature (8 weeks) ARKO mammary glands appeared structurally normal, although the epithelial proliferation (quantified stereologically by PCNA immunopositivity) remained 20% greater than WT (p=0.072). To further characterize the influence of androgens on pubertal mammary growth, the WT and ARKO females were implanted with subdermal depot silastic implants filled with dihydrotestosterone (DHT) at 4 weeks of age and mammary glands collected at 5 weeks of age. DHT significantly (p=0.012) reduced epithelial growth in WT mammary gland but not in ARKO. The mammary gland function appeared affected with reduced weight gain over 15 days from birth for pups from ARKO compared to WT dams. This was supported by a trend for reduced lobuloalveolar differentiation in ARKO mammary gland at day 13 of pregnancy (10.5±1.5 [mean±SD] vs 7.7±1.8 percent of alveoli; stereology). Therefore, the lactational differentiation and post patrium involution processes will be further analysed in detail. In conclusion, the present study provides evidence that AR-mediated androgen actions control mammary growth in a developmental stage-dependent manner and may influence on functional differentiation during pregnancy and lactation.

023

Interleukin (IL)11 mediates protein secretion and modification in human extravillous trophoblasts - identifying a critical role in placentation.

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BACKGROUND: Human trophoblast invasion and differentiation are essential for a successful pregnancy. Dysregulation of these processes can lead to placental pathologies such as pre-eclampsia (PE). The molecular mechanisms of PE are still poorly understood. Interleukin (IL)11, a cytokine shown to negatively regulate trophoblast invasion is possibly involved in the occurrence of PE [1].

AIM: To define the mechanisms by which IL11 regulates the proteome of invasive trophoblasts.

METHODS AND RESULTS: 1st-trimester villous explant cultures showed decreased outgrowth by IL11 treatment ($p<0.05$, n=5). IL11 regulated protein expression was assessed in trophoblastic HTR8/SVneo cells and primary extravillous trophoblasts (EVT). 2D-differential in-gel electrophoresis (DIGE) analysis revealed 731 spots were significantly regulated by IL11 in HTR8/SVneo cells (n=6): 7 spots were analysed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) and 14 unique proteins identified. PDIA3 (protein disulfide isomerase family A, member-3) and GRP78 (glucose regulated protein 78kDa) were further validated as IL11 regulated proteins in HTR8/SVneo and EVT. 1D-Western blot analysis confirmed that PDIA3 was downregulated in EVT ($p<0.05$, n=3). 2D-Western blot analysis revealed that a specific GRP78 isoform was post-translationally modified by IL11 in EVT ($p<0.05$, n=3). Moreover, IL11 stimulated the secretion of GRP78 from EVT ($p<0.05$, n=3) and this effect was abolished by a STAT3 (signal transducer and activator of transcription 3) specific inhibitor.

CONCLUSIONS: IL11 regulated extravillous trophoblast PDIA3 protein expression and secretion/post-translational modification of GRP78 via STAT3 signalling. Moreover, GRP78 was previously identified as a negative regulator of trophoblast motility [2] and that secreted GRP78 could be useful as a predictive marker for PE [3]. Our study demonstrated a unique pathway by which IL11 may direct trophoblast motility and the development of PE.

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024

Temporal Sertoli cell androgen receptor expression and spermatogenic development in immature testis

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The androgen receptor (AR) regulates gene expression in target cells. Androgens are vital for spermatogenesis, yet the precise AR-regulated pathways controlling spermatogenic development remain undefined. Male germ cells do not express AR, highlighting key roles of AR-expressing testicular somatic cells. Sertoli cell AR (SAR) is crucial for postmeiotic germ cell development, yet SAR appears during earlier mitotic expansion and meiotic germ cell development. To determine the role of temporal SAR expression in early postnatal Sertoli and germ cell development, we established a unique transgenic (Tg) SAR mouse expressing human-AR driven via the Sertoli-specific *Abp* promoter. TgSAR was predicted to drive premature postnatal SAR activity. Premature TgSAR was confirmed by confocal immunofluorescence using 5 day-old (do) testes, when WT SAR is low. To identify TgSAR-dependent actions, postnatal TgSAR and WT testes were compared during mitotic (5 do), early and mid meiotic (10 and 15 do, respectively) development. Reported AR-dependent Sertoli-specific transcripts (*Rhox5, Eppin, Amh, Cldn11*) were measured by qPCR. *Rhox5*, *Eppin* and *Cldn11* mRNA were significantly elevated in TgSAR *vs* WT 5 and 15 do testes. Unexpectedly, expression of *Amh,* proposed to be downregulated

by AR, was independent of SAR expression. Sertoli *Dhh* and *Dax1* expression remained normal in TgSAR testes. Mitotic and meiotic germ cell markers were compared in TgSAR and WT testes (qPCR). TgSAR had no effect on expression levels of mitotic (*ckit*) and early meiotic (*Stra8, Piwil2, Sycp1*) transcripts. However, *Dmc1* and *Spo11* transcripts, vital for meiotic pachytene development, were elevated in TgSAR testes. Our novel *gain-of-function* TgSAR strategy revealed that early postnatal SAR regulates the temporal expression of important Sertoli factors, and identified Sertoli transcripts (eg *Amh*) independent of SAR. However, premature SAR did not affect the expression of mitotic or early meiotic germ cell markers, but elevated factors essential for pachytene development. This approach provides a unique opportunity to differentiate AR-regulated genes and pathways during Sertoli and germ cell maturation.

025

Evidence of selective follicular destruction and primordial follicle activation in DMBA induced ovotoxicity

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The environmental xenobiotic 7,12-dimethylbenz-[a]anthracene (DMBA) has a potent ovotoxic affect on rat and mouse ovaries, causing complete follicular depletion resulting in pre-mature ovarian failure (POF). In order to define the molecular mechanisms behind DMBA induced ovotoxicity, we cultured neonatal mouse ovaries in DMBA (50nM) for 4 days and examined its effects on the ovarian transcriptome. Microarray analysis revealed 98 genes were significantly up-regulated >2-fold ($p<0.05$) in response to DMBA exposure, with bioinformatics analysis linking these genes to ovarian signalling pathways associated with follicular growth (mTOR, ILK, VEGF signalling) and atresia (p53 signalling, protein ubiquitination). Validation of our microarray data via qPCR confirmed the up-regulation of several genes ($p<0.05$), including *Cdkn1a* (3.77 fold), *Ddx5* (1.71 fold), *Hspa8* (1.93 fold), *Dnaja6* (2.85 fold), and *Ccnd1* (5.82 fold). Histomorphological and immunohistological analysis supported the microarray data, showing signs of primordial follicle activation, and pre-antral follicle atresia *in vitro* and *in vivo*. Further immunohistological analysis identified significant increases ($p<0.05$) in Akt1 phosphorylation, mTOR activation, and FOXO3a repression in DMBA treated primordial follicle oocytes, events known to be intimately associated with primordial follicle activation. Our results reveal a novel mechanism of DMBA induced pre-antral ovotoxicity involving selective immature follicle destruction and primordial follicle activation via PI3K/Akt and mTOR signalling.

026

Functional characterisation of the GTPase dynamin in mouse spermatozoa

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One of the major unresolved problems of reproductive biology is the elucidation of the key mechanisms that underpin the production of functionally competent spermatozoa that are capable of binding to the zona pellucida and responding to this interaction through the induction of an acrosome reaction. Since the majority of sperm maturational changes occur in the absence of novel protein synthesis they must be largely driven by post-translational modifications and / or remodeling of existing proteins. This has directed our focus towards the potential importance of processes such as intracellular protein trafficking and, in particular, the dynamin family of GTPases. Dynamin exists in three isoforms (Dnm1, Dnm2 and Dnm3) and forms an integral part of the molecular machinery required for vesicle mediated intracellular protein trafficking. Recent research from our laboratory has demonstrated both the presence and cell-specific localisation of the three dynamin isoforms during murine spermatogenesis. Immunofluorescence studies conducted on mouse testis revealed that both Dnm1 and 2 are expressed within a region corresponding to the developing acrosome in maturing sperm whilst Dnm3 appears to reside solely within pre-meiotic germ cells. Dnm1 and Dnm2 are both retained within the peri-acrosomal region of the head of fully mature spermatozoa, and interestingly, upon pharmacological inhibition of dynamin we have observed a significant reduction in the number of sperm that undergo a progesterone-induced acrosome reaction. Additionally, stimulation by progesterone leads to an increase in dynamin 1 phosphorylation, which can be prevented via pharmacological inhibition of dynamin and also by addition of the Ca^{2+} chelator BAPTA. As dynamin activity is well documented to be regulated by changes in Ca^{2+} concentration, we propose the novel hypothesis that, upon progesterone stimulation, dynamin acts to stabilise the numerous membrane fusion events that comprise the mammalian acrosome reaction.

027

Sperm-borne RNA – a novel role in endometrial receptivity for implantation?

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At coitus, seminal fluid interacts with epithelial cells lining the cervix and endometrium to induce expression of proinflammatory cytokines and chemokines, which in turn recruit leukocytes to initiate the immune adaptations required for pregnancy. Signalling factors in seminal plasma including TGF β cytokines have been identified. However recent experiments suggest that sperm also influence female reproductive tract function. Using Affymetrix mouse gene 1.0 ST arrays to compare gene expression in uterine tissue after mating with intact or vasectomized males, we have found that exposure to sperm increases the range and intensity of expression of several inflammation and immune regulation genes compared with seminal plasma alone. We hypothesized that sperm microRNA (miRNA) contributes to these gene expression changes induced by sperm. To examine this, we collected endometrial tissue following mating with intact or vasectomized males after extensive washing to remove superficially-associated sperm. miRNA was extracted and hybridized to Affymetrix mouse GeneChip miRNA arrays, using 4 biological replicates per group. A total of 34 miRNAs were differentially regulated (>1.5 fold) in endometrial tissue after mating with intact males compared with vasectomized males. Of these miRNAs, miR-223 and miR-146a which are involved in the regulation of inflammation were further studied. Using Taqman miRNA assay, a 3.4-fold and 1.9-fold increase in miR-223 and miR-146a respectively was confirmed, and several immune response genes targeted by these miRs were shown to be down-regulated in the predicted fashion. Additionally, Taqman miRNA assay clearly demonstrated that both miR-223 and miR-146a miRNAs were present in sperm purified from the cauda epididymis. The presence of miR-223 and miR-146a in sperm and their subsequent deposition into the endometrial tissue following mating suggests that sperm-borne miRNAs may be novel regulators of inflammation during the peri-conceptual period. Future studies will elucidate the physiological role of these miRNAs using gene silencing techniques.

028

Endometrial epithelial secreted mediator, VEGF enhances embryo development and implantation

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Embryo implantation requires synchronized dialogue between a receptive endometrium and an activated blastocyst via locally produced soluble mediators. During the mid-secretory phase of the menstrual cycle there is increased glandular secretion into the uterine lumen. These secretions contain important mediators that modulate the endometrium and support the conceptus during implantation. VEGF is most well known as a key mediator of vascular growth and remodelling. Previously, using quantitative multiplex analysis we demonstrated the secretion of VEGF into the uterine cavity and further demonstrated a significant reduction in levels of VEGF in uterine lavage fluid from women with unexplained infertility compared to fertile. Functional studies demonstrated that rhVEGF treatment significantly increased the adhesive properties in endometrial epithelial cells (EEC). We hypothesized that VEGF would enhance pre-implantation embryo development and implantation. Swiss mouse blastocysts were treated with media containing rhVEGF (5-500ng/ml) (post-compaction) for 48h. Embryo development was monitored with high temporal time-lapse microscopy, examining blastocoel formation and hatching. Blastocysts were either stained for cell numbers, or transferred to wells coated with fibronectin (Fn) for attachment and outgrowth. Time–lapse microscopy analysis revealed that VEGF treatment enhanced pre-implantation embryonic development. Furthermore, VEGF significantly increased both blastocyst cell number ($P<0.05$) and blastocyst outgrowth ($P<0.05$; 66h on Fn and $P<0.01$ 98h on Fn) compared to control. These findings demonstrate that VEGF plays important roles during embryo implantation. Taken together with the reduction of VEGF in uterine fluid from infertile women and the functional regulation of EEC adhesion, these studies indicate that VEGF is a key factor at the embryonic-maternal interface.

029

Cleavage of endometrial α-integrins into their functional forms at implantation is mediated by proprotein convertase 6

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Proprotein convertases (PCs) post-translationally activate a large number of protein precursors through limited cleavage. PC6 in the human endometrium is tightly regulated during receptivity for embryo implantation. Integrins are transmembrane glycoproteins, some of which play an important role in the adhesive interactions between trophoblast (blastocyst) and uterine epithelium at implantation. For instance, integrin aVb3 is upregulated in the uterine epithelium in humans at the time of endometrial receptivity. We hypothesise that pro-integrin-as in the endometrial epithelium is post-translationally cleaved by PC6 into functional subunits for blastocyst adhesion. To prove this, PC6 was specifically knocked down in an endometrial epithelial cell line, HEC1A by stable transfection with siRNA specific to human PC6 (PC6-siRNA) or scrambled sequence (control). PC6-siRNA cells reduced their capacity to attach to trophoblast spheroids and bind to fibronectin compared to control. Knockdown of PC6 decreased cell surface presentation of functional integrins- α1, α2, α5, αV and αVb5. Western blot analysis demonstrated that PC6 was responsible for the post-translational cleavage of pro-integrin-α5 into its heavy and light chains. Western blot analysis also confirmed the post-translational cleavage of integrin- αV into heavy and light chain. This study demonstrates PC6 as a key regulatory protein essential for the attachment of the blastocyst to the endometrial epithelium through the processing of pro-integrin-as. Compromised PC6 action reduces the post-translational modification of integrin-as thus compromising implantation.

030

The role of ion channels and membrane potential changes at fertilisation in the mouse oocye

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Upon entry into the oocyte, sperm induces a signal transduction pathway resulting in intracellular calcium $[Ca^{2+}]_i$ oscillations and accompanying membrane potential (Em) changes. $[Ca^{2+}]_i$ oscillations have been shown to be important for the developing embryo whereas the role that Em changes play in fertilisation remains unknown. Sulfhydryl reagents such as thimerosal, that oxidise the IP3-R channel, mimic the physiological changes at fertilisation. Patch clamp analysis of unfertilised mouse oocytes has demonstrated that thimerosal elicits simultaneous Em changes and $[Ca^{2+}]_i$ oscillations. We therefore hypothesise that changes in Em following fertilisation may be due to the activation of Ca^{2+} activated Cl^- (CaCC) and K^+ (K_{Ca}) channels present in the membrane of mouse oocytes. The present study aimed to identify these two channels and assessed their role in fertilisation. The thimerosal induced Em changes were inhibited by a CaCC blocker, DIDS, and a K_{Ca} channel blocker tetraethylammonium (TEA), suggesting a role for both CaCC's and K_{Ca} in initiating changes in Em. Removal of Cl^- from the extracellular environment resulted in larger Em changes that continued for a longer period of time as compared to controls. However, the removal of K^+ ions from the extracellular environment inhibited Em changes. These data suggest that a K_{Ca} is responsible for Em oscillations while CaCC's maintain a hyperpolarised Em. To assess the role of a CaCC at fertilisation, *in vitro* fertilisation was performed in the presence of DIDS. DIDS inhibited polar body extrusion and formation of pronuclei. Furthermore, Ca^{2+} imaging experiments showed that DIDS reduced the size, number and duration of Ca^{2+} oscillations induced by thimerosal. Taken together these data suggest that the activation of a Ca^{2+} activated Cl^- and K^+ channel in the mouse oocyte play an important role in initiating the events occurring at fertilisation.

031

Wnt signaling is regulated by leukemia inhibitory factor during mouse embryo implantation.

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Leukemia inhibitory factor (LIF) is an important factor regulating embryo implantation in mammals. In the mouse, LIF is expressed in the glands on morning of day 4 of pregnancy in response to nidatory estrogen. LIF acts on the luminal epithelium via the JAK-STAT pathway, initiating a cascade of events required for creating an environment conducive for embryo implantation. A single injection of LIF to LIF deficient mice on day 4 of gestation can overcome its complete defect in embryo attachment and decidualization. However the downstream signaling and changes in gene

expression induced by LIF in the luminal epithelium are still an enigma. Therefore we devised a model wherein a single dose of LIF was administered to ovariectomized female mice simulated with sequential regimes of estradiol and progesterone to induce a state equivalent to day 4 of pregnancy. Global gene profiling approach in ovariectomized mice treated with LIF for 0, 1, 3 and 6hrs respectively, was able to identify more than 15 sequentially regulated pathways in the luminal epithelium. One prominent pathway identified by this study was Canonical Wnt signaling. Candidate genes identified such as SRY (Sex determining region Y)-box 7 (SOX7), transducin-like enhancer of split 3 (E(sp1) homolog of Drosophila) (TLE3), frizzled homolog 6 (FZD6), WAS/WASL interacting protein family, member 1 (WIPF1) and wingless-type MMTV integration site family, member 7A (Wnt7A) did show alteration in gene expression. E-Cadherin (CDH1), thymoma viral proto-oncogene 1 (AKT1) and beta-catenin (CTNNB1) were also found to be altered by LIF. Sequential analysis of the functions of the genes could help in furthering our knowledge about the signaling events involved in the enigmatic implantation cascade. This research was funded by the Institute of Medical Biology, A*STAR, Singapore.

032

Attenuating sheep oocyte nuclear maturation using cAMP modulation.

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Physical removal of cumulus oocyte complexes (COCs) from the follicle results in spontaneous resumption of meiosis, largely due to a decrease in cAMP levels, resulting in asynchronicity between cytoplasmic and nuclear maturation and decreased oocyte developmental competence. Previously cAMP modulators have been used for the culture of bovine and murine COCs in two stages, pre-in vitro maturation (IVM) and IVM to increase oocyte cAMP concentration to delay meiosis, leading to improved oocyte developmental competence [1] . The aim of this study was to delay meiosis in sheep oocytes by increasing COC cAMP levels using cAMP modulators during pre-IVM and IVM.

Abattoir-derived sheep COCs (age approximately 6-12months), were collected and cultured for 2hr (pre-IVM) in VitroCollect +100µM forskolin (FSK) and 500µM 3-isobutyl-1-methylxanthine (IBMX). Pre-IVM treatment increased COC cAMP levels 10-fold compared to controls ($P<0.05$). Furthermore, the influence of combining pre-IVM (+FSK+IBMX) and 24hr IVM +100mIU/ml FSH + 1µM cilostamide on COC cAMP levels were assessed. During IVM, +FSH increased cAMP by 2.5-fold compared to - FSH (main effect $P<0.001$) and +cilostamide increased cAMP 1.6-fold compared to -cilostamide (main effect $P=0.001$). The combination of pre-IVM (+FSK+IBMX) and 24hr IVM (+FSH+cilostamide) increased COC cAMP levels 2.2 fold compared to standard IVM (-FSK-IBMX/-cilostamide+FSH) and 6.8 fold in systems absent all cAMP modulators (-FSK-IBMX/-FSH-cilostamide). In regards to nuclear maturation +FSK+IBMX/+ FSH+cilostamide delayed completion of meiosis (metaphase II, MII) by 3-4 hours compared to standard IVM, at 21hr +FSK+IBMX/+ FSH+cilostamide was 24% lower in MII rates compared to standard IVM ($P=0.001$) whilst at 24hr the treatments had similar MII rates.

These results demonstrate that a 2 h Pre-IVM phase (+FSK+IBMX) followed by IVM (+FSH+cilostamide), increased COC cAMP levels in sheep and resulted in a delayed, but not inhibited, completion of nuclear maturation. Further work will assess subsequent developmental competence.

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033

IN VITRO generation of bovine pluripotent stem cells and their differentiation towards germ cell lineage.

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Primordial Germ cells (PGC) are the earliest identifiable precursor cell in the germ cell linage. These cells give rise to gametes: mature spermatozoa in male and oocytes in females. Several groups have demonstrated that it is plausible to differentiate pluripotent stem cells such as Embryonic Stem (ES) cells and induced Pluripotent Stem (iPS) cells towards the primordial germ cell lineage [1,2] .This has been possible using different systems of differentiation mainly based on Bone Morphogenetic Proteins (BMPs) stimulus. However, in vitro germ cell competence has been demonstrated only for human, non human primates and mouse ES cell lines. The differentiation potential of pluripotent stem cells generated from domestic species such as *Bos taurus* (cow) remains unknown predominantly due to a lack of pluripotent cell lines in this species. Bovine iPS (biPS) cells were generated using VSV-G envelope-coated pantropic retrovirus for the ectopic expression of human NANOG OCT4, SOX2, KLF4, and c-MYC in bovine adult fibroblast. The biPS cells resemble the characteristics and differential potential of ES cells [3,4] . Subsequently, their *in vitro* germ cell potential was evaluated based on the expression of pre-migratory, migratory and post-migratory markers prior and during differentiation. Embryo body formation by suspension culture and hanging drop method, both accompanied with co-stimulation with human BMP4, were evaluated. Under differentiation, up regulation of markers of pre-meiotic cells such as SYPC3 were identified by RT-PCR. This work on germ cell differentiation from pluripotent bovine cells represents the first steps in a conceptually feasible alternative for germ cells transplantation and the production of sex-selected semen *in vitro*.

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034

The effect of addition of progesterone (with and without removal of initial gonadotrophin) on oestrus synchronisation in pasture-based dairy heifers

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This study evaluated the effect of removing the first GnRH injection or the Progesterone (P4) treatment of a regular GnRH, $PGF_{2\alpha}$, GnRH (GPG) + P4 programme (Prosynch) on synchronisation of ovulation, follicular dynamics and ovulation rate in dairy heifers. In 2009 (n=36) and 2010 (n=38) Friesian and Friesian x Jersey heifers were randomly allocated to three oestrus synchronisation programmes. Group 1 (GPG) received 100 µg gonadorelin (Ovurelin) on day 0, 500 µg cloprostenol, (Ovuprost)on day 7 and 100 µg gonadorelin on day 9, followed by artificial insemination 16 – 20 hrs later. The programme for Group 2 (GPG+ P4) was the same as Group 1 with the addition of a P4 releasing intravaginal device (Cue-Mate) from day 0 to day 7. Group 3 (PG+ P4) was treated the same as Group 2, except for the absence of the first gonadorelin treatment. Ultrasonography was performed on days -2, -1, 0, 1, 2 3 and 7 and then at 12 hourly interval on days 9 to 11. The mean diameter of the largest follicle on Day 1 (P =

0.02) and Day 3 (P = 0.04) was significantly larger in the PG+ P4 group compared to the GPG+ P4 group. On Day 2 the mean diameter of the PG+ P4 group was significantly larger (P=0.02) compared to the GPG and the GPG+ P4 group and the emergence of a new follicular wave was significantly longer (4.33 vs. 2.97 days, P = 0.03) in the PG+ P4 group compared with the combined GPG (2.67 days) and GPG+ P4 (3.2 days) group (with first GnRH). However this alteration in follicular dynamics at the start of the programme had no influence on the proportion of heifers which ovulated within 48 hours of the last gonadorelin injection (0.81, 0.84 and 0.81 in GPG, GPG+P4 and PG+P4 respectively) or in the timing of ovulation within that period (P=0.86)

035

Musashi-2 is responsible for the translational control of *m-numb* in mouse oocytes: a potential role for RNA-binding proteins in meiotic spindle assembly

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Control of the maternal mRNA pool during oocyte maturation is crucial to the correct temporal and spatial expression of proteins, particularly during oocyte transcriptional quiescence. We have identified the Musashi (Msi) family proteins, Msi-1 and Msi-2, within the mouse oocyte/ovary, where the RNA-binding proteins are believed to act as translational control proteins.

Using Msi-1 protein-RNA immunoprecipitation, we have previously identified *musashi-2* (*msi-2)* as a putative target in the mouse oocyte. To study these targets, a transgenic mouse was produced to overexpress Msi-1 exclusively in the oocyte. Overexpression of Msi-1 in the target tissue has been confirmed via QPCR analysis and immunoblotting.

QPCR analysis of Msi-1 target expression performed on transcripts obtained from the Msi-1 protein-RNA immunoprecipitation, revealed a ~55-fold enrichment of *msi-2* transcript above control IP levels. Similar analysis performed on intact ovaries of tgMsi-1 and WT mice, showed a 7-fold increase in *msi-2* expression in tgMsi-1 ovaries. This suggests that *msi-2* mRNA is translationally repressed by Msi-1.

Preliminary Msi-2 protein-RNA immunoprecipitation studies have revealed Msi-2 to target transcripts encoding crucial proteins involved in cell cycle regulation and cell fate decisions, with *cdkn1c* enriched ~64-fold and *m-numb* and *mtf2* enriched ~7-fold above control levels. Recent studies have shown m-Numb, in particular, to play a crucial role in meiotic spindle assembly during the oocyte re-entry into meiosis.

To further investigate the role of Msi-2 in mouse oogenesis, an oocyte-specific tgMsi-2 overexpression mouse has been generated, with preliminary studies suggesting that the ovaries of tgMsi-2 mice are smaller than their WT littermates. Functional quantification of oocyte development demonstrates more oocytes are produced from superovulated juvenile tgMsi-2 mice than their WT littermates.

Therefore, Msi-2 may play a crucial role in oocyte development and this may act via targeting *m-numb* for post transcriptional control.

036

Diet and exercise improves sperm function in obese mice

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Paternal obesity is associated with reduced sperm motility, morphology and increased sperm DNA damage and oxidative stress impairing sperm function and subsequent pregnancy and offspring health. However, currently there is no information as to whether this effect can be reversed by weight loss. Therefore the aim of this study was to assess the reversibility of obesity induced perturbations on sperm function through weight loss via diet and/or exercise interventions.

C57BL6 male mice (n=20) were fed either a control diet (CD) (6% fat) or a high fat diet (HFD) (21% fat) for a period of 10 weeks before HFD mice were allocated to either (i) continuation of a HFD (HH), (ii) a HFD with swimming exercise (HE), (iii) changed to a CD (HC) and (iv) a CD with swimming exercise (HCE) for a further 8 weeks. CD mice continued with the CD for the same period. Sperm were collected post mortem from the cauda epididymidis for analysis of motility, morphology, capacitation, sperm binding, mitochondrial metabolism, DNA damage and reactive oxygen species.

Both groups of mice receiving diet interventions (HC and HCE) had significant reductions in total body weight and adiposity compared with obese mice (HH) (-40%, $p<0.05$) with the exercise only group (HE) maintaining both their level of adiposity and total body weight. Basic sperm parameters including sperm motility, morphology and capacitation were significantly improved in all intervention groups (+16%, +17% and +12%, $p<0.01$). Significant reductions were also seen in sperm DNA damage, reactive oxygen species and mitochondrial membrane potential (-63%, -12% and -18%, $p<0.05$) which lead to significant increases in the functional measure of sperm binding (+66%, $p<0.05$) in both diet and exercise treated mice.

This is the first study to show that the abnormal sperm physiology resultant from obesity can be reversed through diet and exercise, highly suggestive that diet and lifestyle interventions could be utilised to reduce the sub fertility associated with obesity.

037

Lack of a Fanconi Anemia DNA repair pathway in oocytes during meiosis may make them susceptible to interstrand crosslinking during prophase I arrest

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DNA repair is important for maintaining genomic integrity and preventing chromosomal abnormalities that lead to aneuploidy. Different types of DNA damage induce distinct repair pathways. One important mitotic repair mechanism involves the Fanconi Anemia (FA) pathway, which recognises and repairs DNA cross-links. Deficiencies in this pathway result in DNA damage accumulation, which causes chromosomal aberrations. Oocytes spend majority of their lives arrested at the dictyate stage of prophase I, however, little is known about their capacity to repair damaged DNA. Therefore, we examined FANCD2, a core component of the FA pathway, in mature mouse oocytes and cultured mitotic cells. We observed that in HeLa cells FANCD2 localised to the nucleus during S-phase as a result of DNA repair being initiated upon replication, which is a well-known

phenomenon. Upon addition of 0.75μM Mitomycin C (MMC), a chemotherapeutic agent which induces cross-links, the amount of activated FANCD2 foci per nuclei was significantly increased, from 18 +/- 13 foci to 49 +/- 23 foci ($P<0.001$, student's t-test, n=161, 187). In contrast, prophase I arrested oocytes did not show any FANCD2 nuclear staining, even with 9μM MMC. Following meiotic resumption, FANCD2 immunofluorescence revealed a spindle microtubule localisation during meiosis I and II, unlike an association with chromosomes observed in mitosis. Additionally, doses as high as 60μM did not decrease rates of oocyte maturation. Interestingly, only when oocytes had completed meiosis and embryos had entered the mitotic cell cycles, did FANCD2 localise to the nucleus. In conclusion, the above observations suggest that oocytes do not repair DNA through the FA pathway until meiosis has been completed and the embryonic cell cycle initiated. We surmise that the apparent lack of DNA repair during meiosis would make the oocyte sensitive to cross-linking DNA damage, consequently leading to aneuploidy and infertility.

038

The Endometrium: Debut into the Stem Cell World

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The discovery of human embryonic stem cells heralded a new field of regenerative medicine. It energized adult stem cell research, with a broadening of scope to include organs of low cell turnover as well as highly proliferative tissues. Endometrium is highly regenerative, undergoing >400 cycles of growth, differentiation and shedding during a woman's reproductive years. Our aim was to identify adult stem/progenitor cells in human and mouse endometrium. We discovered rare epithelial and stromal colony forming cells/units (CFU) in human endometrium and demonstrated their ability for self renewal, proliferation and differentiation. Epithelial progenitors differentiated into gland-like structures, and stromal CFU into smooth muscle, fat, bone and cartilage lineages, indicating their mesenchymal stem/stromal cells (MSC) phenotype. Mouse endometrial stem/progenitor cells were identified as label retaining cells (LRC), which can drive estrogen-stimulated regeneration. We identified novel surface markers showing promise for purifying epithelial stem/progenitor cells. Our markers for endometrial MSC (eMSC) demonstrated their perivascular location and likely pericyte identity. EMSC are present in both the functional and basal layers of human endometrium and are shed during menstruation. Preliminary findings show that cells expressing endometrial stem/progenitor cell markers are present in peritoneal fluid of menstruating women, suggesting their possible role in initiating endometriosis lesions. Endometrial cancer harbors rare cancer stem cells capable of producing tumours recapitulating histoarchitecture and differentiation of parent tumours. We are now investigating the use of eMSC in a tissue engineering construct as a novel therapeutic for pelvic organ prolapse repair. These fundamental studies allow us to examine the role of endometrial stem/progenitor cells in the pathogenesis of gynecological disorders associated with abnormal endometrial proliferation; endometriosis, adenomyosis and endometrial cancer. Conversely, defective function or lack of endometrial stem/progenitor cells may result in an inadequate endometrium for embryo implantation.

040

Intra-ovarian factors regulating thecal androgen production

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Theca cells have an indispensible role in follicle development and function, providing structural support and being responsible for de novo synthesis of androgens that are essential substrates for oestrogen synthesis by neighbouring granulosa cells. Both androgens and oestrogens influence many physiological processes in the female and their actions extend well beyond maintaining and coordinating cyclical changes at the level of the ovary and reproductive tract. Dysregulation of thecal androgen synthesis is associated with various diseases including the widespread polycystic ovarian syndrome. Whilst pituitary LH is long recognised as a key driver of theca cell function, in vitro studies indicate that many other systemic (e.g. insulin, IGF, leptin) and intra-ovarian (e.g. SCF, IGF, EGF, FGF, TGFβ, activin, inhibin, BMP, GDF, oestrogen) signalling factors have important modulatory roles, with intra-ovarian signals emanating from granulosa cells, oocyte, theca and stroma. The principle experimental model used in this laboratory utilizes primary bovine theca interna cells cultured under defined serum-free conditions. In this presentation the focus will be on recent evidence indicating (1) autocrine/paracrine regulation of androgen production by several members of the TGFβ family including BMPs and activins; (2) modulation of BMP/activin signaling by locally produced binding proteins; (3) modulation of BMP/activin signalling by inhibin; (4) an association and possible functional link between thecal INSL3 and inhibin α subunit expression and the maintenance of androgen production. The picture that emerges is highly complex with an interacting network of positive and negative signals, the prevailing balance of which presumably dictates the level of androgen output. Supported by BBSRC

041

Macrophage Regulation of Corpus Luteum Angiogenesis in Early Pregnancy

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Macrophages have been implicated in tissue remodelling and angiogenesis in the corpus luteum, but their precice function is unclear. This study utilised *Cd11b*-diphtheria toxin receptor *(Cd11b-Dtr;* Tg(ITGAM-DTR/EGFP)34Lan) transgenic mice to elicit acute transient depletion of macrophages by administration of diphtheria toxin (DT). Previously we reported that macrophage depletion during the pre- and peri-implantation phase of pregnancy caused complete pregnancy loss in DT-treated *Cd11b-Dtr* mice. This was associated with reduced serum progesterone (P_4) in *Cd11b-Dtr* mice and pregnancy could be rescued by administration of exogenous P_4 [1]. Histological analysis of ovaries from DT-treated *Cd11b-Dtr* mice showed evidence of functional and structural luteolysis with disruption of the microvascular network within corpora lutea. In the current study, we analysed expression of the *Vegf* genes and their receptors in ovaries by real-time PCR on day 1.5 and 2.5 pc, 24 h following macrophage depletion. A 3.6-fold or greater increase in *Vegfa* mRNA expression was seen in macrophage-depleted mice, but a significant decrease in *Vegfc* (41.2% and 47.5% reduction on days 1.5 and 2.5 pc) and *Figf (Vegfd*; 30.2% and 28.6% reduction on days 1.5 and 2.5 pc). *Flt1 (Vegfr1)* showed a 53.4% decrease following macrophage depletion, and *Kdr (Flk-1;Vegfr2)* revealed a 33.3% decrease. Macrophages are reported to synthesise VEGFC and FIGF [2] , which are best known for their role in lymphangiogenesis, but can also activate KDR and induce blood vessel growth. Endothelial cells express FLT-1 and KDR and monocytes and macrophages express FLT-1; the decrease in *Vegf* receptor gene expression is consistent with absence of these cells from CLs in DT-treated *Cd11b-Dtr* mice. In conclusion, these data indicate a critical role for macrophages in the provision of trophic support for the microvasular network in the corpus luteum during early pregnancy. Dysregulation in macrophage-regulated CL development may contribute to some forms of unexplained infertility.

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042

Cumulus cell EGF-like peptide and receptor signalling during oocyte *in vitro* maturation

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EGF-like peptide signalling mediates the ovulatory signal, leading to the propagation of several cellular events that elicit the ovulation of a competent oocyte. Although the function and impact of EGF-like peptide signalling during *in vivo* oocyte maturation has been recently characterised, little is currently known about the effect of oocyte *in vitro* maturation (IVM) on this pathway. We examined EGF-like peptide signalling in cumulus cells during IVM. Time course experiments (0-12h) showed that while there were no differences in *Egfr* mRNA expression in cumulus cells between IVM and *in vivo* maturation, mRNA expression of the 3 EGF-like peptides was absent for the majority of IVM and significantly lower than *in vivo* ($p<0.01$). We then examined the effect of routine IVM additives: FSH +/- EGF and forskolin- on cumulus cell *Egfr* and EGF-like peptide mRNA expression, as well as EGFR phosphorylation. Under control conditions (no additives), there was no EGF-like peptide expression after 6-12h and no EGFR phosphorylation, despite high levels of *Egfr* expression. Treatment of COCs with forskolin, FSH, EGF or FSH+EGF lead to a dramatic increase in expression of all 3 EGF-like peptides during early IVM (2-6h), with forskolin most potent (30-3607 fold at 2h), followed by EGF (77-2015 fold), FSH+EGF (52-1187 fold) and FSH (20-80 fold) at 6h. Expression levels of the 3 peptides fell by 12h in all treatments but were still significantly higher than control. FSH +/-EGF treatment led to transient phosphorylation of the EGFR (0.25-3h) followed by notable down-regulation of *Egfr* mRNA (6-fold relative to control). Expression of the EGF-like peptides, but not the *Egfr*, is perturbed during IVM. This can be reversed by forskolin or EGF. This provides insight into the functioning of a crucial signalling pathway during oocyte IVM and hence opportunities to modulate it to improve the efficiency of clinical IVM.

043

The potential role of the chemotactic signalling axis, CXCR4/CXCL12, during ovulation

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Ovulation requires a specific sequence of molecular events for the successful release of a fertilisable oocyte. These events are not entirely understood, but the progesterone receptor (PGR) plays an essential role, as $PR^{-/-}$ mice are completely anovulatory, despite normal growth and development of ovarian follicles and oocytes (1, 2). Our microarray study, to identify PGR-regulated genes in the ovary and oviduct, identified the chemokine receptor, *Cxcr4*, to be >3-fold down-regulated in granulosa cells (GCs) from ovaries of $PR^{-/-}$ mice at 8h post-hCG. *Cxcr4* is known to regulate immune cell chemotaxis (3) and cancer cell invasive migration (4). We recently discovered that the cumulus oocyte complex (COC) displays a transient adhesive, migratory and matrix-invading phenotype at ovulation and are currently examining potential mechanisms that induce this behaviour. We hypothesised that CXCR4 and its ligand CXCL12 may play a role in signalling between the periovulatory COC and the oviduct at ovulation. We found a dramatic (>50-fold) induction of *Cxcr4* mRNA by luteinising hormone (LH), peaking at 8h post-hCG. We also confirmed that *Cxcr4* is significantly down-regulated in ovarian GCs and COCs from $PR^{-/-}$ females relative to $PR^{+/-}$ littermates. The ligand, *Cxcl12*, was not regulated by PGR or LH but was constitutively expressed at high levels in the oviduct relative to the ovary (10X GCs and COCs). Similar expression patterns were found by immunohistochemistry for CXCR4 and CXCL12 in ovaries and oviducts. However, treating mice with the CXCR4 inhibitor, AMD3100, did not affect in vivo ovulation rates. Further, CXCL12 did not induce migration of preovulatory COCs in vitro. Interestingly, migration of COCs was significantly impaired by a high dose (400ng/ml) of CXCL12. We conclude that while the pattern of expression of this chemokine signalling axis in the ovary and oviduct during the periovulatory period is compelling, its functional significance requires further investigation.

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(3) Bleul et al, 1996, J Exp Med
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044

Circadian variation in placental expression of inflammatory mediators

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Rhythmic expression of clock genes drives circadian variation in various physiological processes both centrally and in peripheral tissues such as the liver. We recently demonstrated the rat placenta expresses all canonical clock genes in a highly zone-specific pattern and with some circadian variation. Placental inflammatory status influences the onset of parturition, the timing of which shows a distinct circadian pattern in the rat, and systemic levels of pro-inflammatory cytokines also exhibit circadian variation. Therefore, we tested the hypothesis that placental expression of inflammatory mediators varies in a circadian manner.

Pregnant rats (n=6/time point) were sampled over days 21-22 of gestation (term = day 23). Samples of junctional (JZ) and labyrinth (LZ) zones of the placenta were collected at 0800, 1400, 2000 and 0200 h, which equate to zeitgeber times ZT1, ZT7, ZT13 and ZT19 respectively. JZ and LZ expression of mRNA encoding pro-inflammatory mediators (*TNF-α, IL-6, IL-1*β , *Cox1* and *Cox2*) and an anti-inflammatory circadian regulator (*Sirt1*) were measured by RT-qPCR.

LZ expression of *TNF-α, IL-6, Cox2* and *Sirt1* all varied with time-of-day. Specifically, peak *TNF-* α expression at ZT1 was 2.2-fold higher than its trough at ZT13 (P=0.034). *IL-6* expression was 2.3-fold higher at ZT19 than at ZT7 (P=0.004), while *Cox2* expression at ZT13 was 2-fold higher than at ZT7. *Sirt1* expression at ZT13 was 1.6-fold higher than at ZT1 (P=0.05). Circadian variation was less prevalent in the JZ, although TNF-α and IL-1β expression were 1.6-fold (P=0.021) and 2.4-fold (P=0.038) higher, respectively, at ZT19 than at ZT7.

In summary, these data show that the placenta exhibits circadian variation in expression of pro-inflammatory mediators late in gestation, with cytokine expression highest late in the active (dark) phase and early in the inactive (light) phase. This circadian pattern of placental inflammatory status is likely to influence placental physiology and the timing of parturition.

045

The molecular control of reproduction

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Marsupial mammals diverged from eutherian mammals over 130 million years ago, but share the same mechanisms that regulate reproduction and development. Many genes have a expression pattern that is highly conserved between mouse and marsupial during gonadal development. For example, FGF9 protein is detected in the seminiferous cords of the developing tammar testis in the Sertoli and germ cells, while WNT4 is localised in the ovarian cortex. This suggests that FGF9 promotes male fate in the early gonad of marsupials through an antagonistic relationship with WNT4, as it does in eutherian mammals. In contrast, there are several developmental genes that have not yet been analysed in adult eutherians. For example, mutation of the *Kallmann syndrome 1* gene (*KAL1*) or *X-linked aristaless homeobox* gene (*ARX*) in humans leads to a dysfunctional testis, but there has not been any direct evidence to explain the failure of spermatogenesis. In the tammar adult testis, *KAL1* mRNA is present in round spermatids and elongated spermatids. In the adult ovary, the protein was detected in the oocytes and granulosa cells. This demonstration that *KAL1* mRNA and protein are expressed in adult mammalian gonads suggests that, in addition to regulating reproduction through the hypothalamic-pituitary-gonadal axis, *KAL1* may work at a local level in the gonads themselves to regulate spermatogenesis and folliculogenesis. Similarly, tammar *ARX* has an expression pattern in brain and gonadal development that is highly conserved with that of man and mouse, but there are no studies of the adult gonad. In the tammar, it is specifically expressed in the round spermatids. We also detected *Arx* in the mouse round spermatids. Thus it appears that ARX is involved in spermatogenesis in mammals as well as in the developing brain and gonad, and like *KAL1*, has previously unsuspected functions in mammalian spermatogenesis.

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046

Transcriptional and chromatin regulation during male fetal germ cell differentiation

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Germ cells convey genetic and epigenetic information to the following generation. During early germ line development the germ cells undergo extensive epigenetic reprogramming that clears the way for establishment of the male and female germ cell epigenomes. Sex specific germ line development is initiated in the mid-gestation embryo. In males germ cells commit to the spermatogenic pathway and enter mitotic arrest, whereas in females they immediately enter meiosis. In some circumstances germ cell differentiation is compromised, pluripotency is reactivated and germ cell tumours can result. We have conducted gene expression profiling of germ cells from embryonic day (E)12.5 - E15.5 male and female gonads, encapsulating early germ cell differentiation. Using biological contextualization tools we have focused on a subset of genes that are specifically regulated in male germ cells, conserved in human and mouse and have been associated with cancer, chromatin / epigenetic regulation and control of cell cycle. To examine chromatin regulation during male germ cell development we have designed high-density custom microarrays and conducted ChIP-on-chip experiments for activating (H3K4me3) and repressive (H3K27me3) histone modifications on the promoters of a subset of these male germ cell identity genes. Our preliminary analyses indicate that many genes are enriched for H3K4me3 at E13.5 and E15.5. By contrast we identified only two genes enriched for H3K27me3 marks at E13.5. However at E15.5 a further 11 genes had recruited H3K27me3. Surprisingly these genes were almost exclusively transcriptionally activated despite recruitment of this repressive mark. To extend these finding we are currently examining the role of polycombe repressive complex proteins in establishment of epigenetic patterning of the male germ line. Further understanding of these processes promise to lead to greater insight into the fundamental molecular mechanisms underlying male germ line differentiation and potentially the initiation of germ cell-derived testis tumours.

047

Differential regulation of foetal mouse testis somatic cell markers by TGFβ2

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Gonadal development is a complex process involving both somatic and germ cells. Aberrant development of either cell type leads to clinical pathologies, such as infertility and cancer. We have localised betaglycan (TGFBR3), the TGFβ family co-receptor, to the somatic cells of foetal mouse testis (1). Analysis of the betaglycan knockout mouse testis demonstrated that absence of betaglycan leads to defects in seminiferous cord formation and in Leydig cell development (2). As betaglycan is particularly important for TGFβ2 action, we hypothesised that somatic cell development depends upon TGFβ2 in the foetal testis. We tested this hypothesis, using a gonad explant culture system to analyse the effects of exogenous TGFβ2 on wildtype and betaglycan null testis. Gonads from 13.5 dpc embryos were cultured for 48 hours, with and without recombinant TGFβ2 (2.5-5 ng/ml). Real Time quantitative PCR analysis and immunohistochemistry were then used to assess changes on the gene expression and cellular protein levels, respectively. TGFβ2 treatment of wildtype testes significantly down-regulated the Sertoli cell marker *Amh* at the mRNA level (65% $p<0.001$) while *Sox9* was unaffected, suggesting TGFβ2 acts downstream of *Sox9*, directly regulating *Amh* in the foetal mouse testis. Treatment of the wildtype testis also differentially affected Leydig cell markers. Genes which regulate steroidogenesis (*Sf1, Star, Cyp11a, Hsd3b1* and *Cyp17a1*) were significantly downregulated (43-74.4%; $p<0.0001$) while *Insl3*, another Leydig cell marker, was not. In betaglycan null testis, TGFβ2 treatment partly rescued cord formation; however, TGFβ2 did not further reduce the existing low expression of Leydig cell genes in betaglycan null testis. These data indicate that TGFβ2 negatively regulates a subset of somatic cell genes in foetal murine testis and acts via betaglycan to regulate cord formation. Low expression of somatic cell markers in betaglycan null testis may indicate a block in somatic cell differentiation, upstream of TGFβ2-mediated effects on steroidogenesis.

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048

Morphological and molecular development of the prostate in the tammar wallaby, *Macropus eugenii*

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The tammar prostate has a single lobe surrounding the urethra, with distinct anterior, central and posterior zones, which is morphologically more similar to the human prostate than the classically studied model, the mouse. This study characterised the morphological development of the tammar prostate, and the expression of several markers of prostatic bud induction that are important in eutherians, namely FoxA1, SOX9 and NKX3.1. The urogenital system (UGS) of early stage male and female tammar pouch young is a smooth tube of urogenital epithelium surrounded by mesenchyme. The first prostatic buds appeared in males by day 25 postpartum. These prostatic buds developed into solid prostatic cords that projected into the outlying mesenchyme. By day 60 postpartum, there was evidence of lumen formation within the solid cords of epithelium which joined to the central urogenital lumen. Cellular proliferation (as assessed by detection of proliferating cell nuclear antigen, PCNA) occurred at the distal tips of prostatic buds. Tammar homologues of the marker genes FoxA1, SOX9 and NKX3.1 were cloned. FoxA1 and SOX9 had high homology with the human sequences. NKX3.1 was highly conserved within the NK box region but exon 1 could not be found, consistent with high divergence of this exon in other mammals. The pattern of expression of NKX3.1 could not be assessed as none of the antibodies tested were effective. FoxA1 and SOX9 were expressed in the urogenital tissues and protein was detected in the nuclei of urogenital epithelial cells in both males and females. The pattern of expression was similar to that seen in developing humans. These results indicate that both morphological and molecular development of the tammar prostate is highly conserved between the tammar and human. The tammar may therefore be a useful model species for understanding the pathways involved in prostatic morphogenesis.

049

Monotremes provide a key to understanding the evolutionary significance of epididymal sperm maturation

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It has been widely accepted that mammalian spermatozoa are infertile when they leave the testes and require a period of maturation in the specific milieu provided by both the epididymides and the female reproductive tract before acquiring the ability to fertilise an oocyte. The necessity for such a complex process of post-testicular sperm maturation appears to be unique to mammals since it is well established that these processes are of little significance to the spermatozoa of birds, reptiles and other lower vertebrates. However, whether the sperm of monotremes (echidna and platypus), the earliest off-shoot of the mammalian lineage, require post-testicular maturation in order to become fertile has not been established, therefore questioning the validity of this dogma to all mammals. Moreover, due to their key evolutionary position and form of reproduction, monotremes provide a unique model for resolving how and why these processes are necessary. The aim of the present study was therefore to characterise the processes involved in epididymal maturation and capacitation of monotreme spermatozoa. Our studies have demonstrated that monotreme sperm maturation is far less complex than in other mammals. However, the monotreme epididymis is unique in forming spermatozoa into bundles of approximately 100 with greatly enhanced motility compared with individual spermatozoa. Sperm bundle formation involves a highly organised interaction with epididymal proteins, and the bundles persist for 2-3 h during incubation in vitro, before dispersing spermatozoa that have the capacity to bind the avian perivitelline membrane in a heterologous sperm adhesion assay. It is suggested that the unique co-operative strategy employed by monotreme sperm represents an early form of epididymal maturation. Furthermore, it appears that this process has since been elaborated upon during the evolution of higher mammals, possibly as an adaptation for sperm competition, that is, the competition between males to achieve paternity.

050

Environmental Effects on Fetal Reproductive Development

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It has long been know that the environment can influence mammalian reproduction. There have been difficulties obtaining robust data. In the last 6 decades there has been a growing awareness of the link between environmental chemicals (ECs), especially those with Endocrine-Disrupting activity (EDCs) and reduced reproductive performance in wildlife and domestic animals and our own species. Descriptive syndromes have been coined to describe some of these effects: Testis and, more recently, Ovary dysgenesis syndromes (TDS, ODS). The key concept of these syndromes is that environmental exposures disturb one or more aspects of fetal gonadal development in-utero, leading to an increased incidence of gonadal and other defects, such as hypospadias in males or advanced menopause in females. Cigarette smoking provides a unique model to study human endocrine disruption: up to 30% of women are smokers and few give up when pregnant. Cigarette smoke contains >4,000 pollutant chemicals, including potentially toxic elements (PTEs, e.g. cadmium) and persistent organic pollutants (POPs, e.g. dioxin), many of which are also EDCs. This presentation will summarise existing knowledge of the in-utero effects of smoke-exposure and review both published and emerging data from our studies of elective terminations of normal second trimester human fetuses. In reproductive terms, the outcomes of in-utero exposure to cigarettes are similar between the two sexes. In men these include: reduced testis size, reduced sperm counts, reduced sperm quality, increased incidence of hypospadias & cryptorchidism, increased free testosterone, reduced inhibin B, earlier puberty onset, increased BMI, reduced height. In women these include, reduced fecundity, earlier menarche and menopause onset, increased BMI, reduced height. Our own work shows that gonadal genes, liver polycyclic aromatic hydrocarbon (PAHs: AhR-inducers) burden and liver enzymes are affected if the mother smokes cigarettes while pregnant. The take-home message is that exposure to chemical cocktails does affect human reproductive development. Much of this work is supported by the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement no 212885.

051

Diet induced paternal obesity in the absence of diabetes impacts upon developmental and neuromuscular outcomes in neonate offspring in mice.

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We recently reported that paternal exposure to a high fat diet (HFD) resulting in obesity in the absence of diabetes adversely affects offspring's metabolic and reproductive health. Other paternal characteristics, notably age, are associated with behavioural changes and increased risk of schizophrenia or autism in children, suggesting a paternal influence on neurological outcomes in offspring. The relative contribution of genetics or paternal environment is difficult to delineate in human cohorts.

Here we show that paternal exposure of C57BL6 mice to a HFD (21% fat) for 8 weeks also impaired developmental and neuromuscular outcomes in neonate offspring. HFD founder sired pups (n=38) exhibited precocious eye opening (-1.14 days P=0.04), precocious pinnae (ear) detachment (-0.28 days P=0.02) and reduced grip strength in a wire hang test when compared to pups sired by control diet (CD; 6% fat) founders (n=64). Interestingly male pups sired by a HFD founder had heightened tactile orientation responses when compared to pups from a CD founder, but this was not observed for female pups. Conversely female pups from a HFD founder had sensitised palm reflexes that were not observed for male pups.

The reduced grip strength of pups from a HFD founder may result from increased pup body weight (male +6.2% P=0.005; female +7.9% P=0.008) without a proportional increase in muscle strength, or as an early indication of glucose intolerance (area under the curve (AUC); male +23.0% P=0.05; female +11.1% P=0.02) and insulin resistance (AUC; male -30.5% P=0.04; female -43.6% P=0.02) observed in these animals in adulthood.

This demonstrates that a paternal HFD influences developmental and neuromuscular outcomes in neonate offspring with observed sex differences. Offspring muscle weakness may contribute to obesity and insulin resistance in this generation and neuromuscular deficits warrant further investigations surrounding any long term consequences of paternal obesity on offspring neurological function and behaviour.

052

13-cis-retinoic acid Induces DNA Damage in the Male Germ Line

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13-cis-retinoic acid is a vitamin A derivative commonly prescribed by dermatologists as an effective treatment for moderate to severe acne. To determine the effects of this drug on human spermatozoa, *in vitro* dose and time dependent assays using 13-cis-retinoic acid were conducted. These assays revealed a significant percentage of spermatozoa generated superoxide anion almost immediately. This was detectable in the mitochondria using MitoSOX Red, a mitochondrial superoxide anion probe ($p<0.0001$). Superoxide anion was also detected within the cytoplasm by dihydroethidium at doses as low as 12.5µM. Within 1 hour a highly significant population of spermatozoa were immotile and had lost mitochondrial membrane potential ($P<0.0001$). Reactive oxygen species induced peroxidation of membrane lipids was observed at doses of 25µM and cell viability was decreased at this dose. The formation of 8-hydroxy-2'deoxyguanosine was detected using a FITC-conjugated antibody against this highly mutagenic oxidative DNA lesion, as was DNA strand breaks measured by the TUNEL assay. DNA damage in the male germ line is correlated with poor pregnancy rates, increased miscarriage and morbidity in the offspring. Considering these implications, further research into the *in vivo* effects of this drug should be undertaken.

053

Paternal diet-induced obesity retards early embryo development, metabolism, and quality

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In Australia, 68% of the adult male population is overweight or obese. An association between infertility and excessive body weight is now accepted, though focus remains primarily on females. Previously we demonstrated that parental obesity resulted in compromised embryo development, disproportionate changes in embryo metabolism and reduced blastocyst cell number. Here we hypothesise that paternal obesity negatively impacts sperm, resulting in compromised embryos. This study used in vitro fertilisation (IVF) to isolate the effects of paternal obesity on sperm; and subsequent embryo quality and implantation.

Epididymal sperm was collected from normal and obese C57BL/6J mice and cryopreserved for subsequent IVF with oocytes collected from Swiss females (normal diet/weight). Generated embryos were cultured individually; development was monitored with high temporal time-lapse microscopy. At 36h post-fertilisation, mitochondrial membrane potential and pyruvate uptake of the 4-cell embryo was quantitated. At 92h post fertilisation, glucose utilisation and lactate production of blastocysts was determined using ultramicrofluorimetry and followed by either differential cell staining of the trophectoderm (TE) and inner cell mass (ICM), blastocyst outgrowth on fibronectin or transferred to recipients for implantation.

Paternal obesity caused a significant delay in preimplantation embryo development ($P<0.05$). Metabolic differences observed were associated with a reduction in mitochondrial membrane potential ($P<0.05$). Differential staining revealed significant differences in the ratio of cell allocation to TE and ICM lineages ($P<0.05$). Blastocysts generated from obese sperm displayed significantly reduced outgrowth ($P<0.05$) and decreased fetal developmental following embryo transfer ($P<0.05$). These findings confirm an effect of paternal obesity on embryo health and pregnancy quality.

054

The consequences of chronic acrylamide exposure on the male germ line

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Acrylamide is a reproductive toxicant that has been detected in cooked, carbohydrate-rich foods such as potato chips and breads. The consequences of chronic exposure to acrylamide in the human diet are unknown, however rodent experiments have shown that acute acrylamide exposure in males can lead to decreased fertility, reduced litter sizes and an increase in embryo resorptions. The mechanism by which acrylamide elicits these effects is thought to be related to its metabolic conversion to glycidamide, which can cause genetic damage through the formation of DNA adducts. Previously, we found that in vitro acrylamide exposure induced glycidamide-DNA adducts in isolated male germ cells of mice. To determine whether chronic acrylamide exposure could produce similar genetic damage in male germ cells in vivo, male mice were subjected to acrylamide through the drinking water at doses relevant to human levels of exposure (between 0.001 and 10µg/ml) for up to 12 months. Testis histology was examined at 1, 3, 6, 9 and 12 months and male germ cells were measured for DNA damage using a Comet assay modified to detect adducts. Acrylamide treatment did not significantly affect mouse weight or testis weight and no gross morphological effects were observed in the testis. However, a significant time and dose-dependent increase in adduct-related DNA damage was observed in germ cells. Six months of exposure to the two highest doses (1 and 10µg/ml) was sufficient to produce a response. After 12 months of exposure, significant increases in damage were detected at doses as low as 0.01µg/ml. These results demonstrate that chronic dietary exposure to acrylamide can generate DNA damage in male germ cells. This study is the first to demonstrate the consequences of xenobiotic exposure in the male germ line at chronic levels.

055

Microtubule severing and male fertility

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Microtubules are a component of the majority of cell types and play essential roles in processes including motility, division and cell shaping. In turn microtubule dynamics is controlled by a number of pathways including those that can rapidly polymerize or destabilize ends to change microtubule length, or cut microtubules to provide seeds capable of being transported around a cell for later extension. Using a random mouse mutagenesis screen we have produced the 'Taily' mouse line. Male Taily mutant mice carry a point mutation in the WD40 region of the microtubule severing gene/protein KATNB1 (p80 katanin). Katanin is an evolutionarily-conserved microtubule severing complex that is emerging as a key regulator of microtubule dynamics in lower order species, where it has been implicated in the regulation of microtubule growth, length and number. The katanin complex is composed of a p60 severing enzyme and a p80 regulatory subunit that modulates katanin targeting and activity. We show that homozygous p80/*Katnb1* mutant male mice are sterile as a consequence of decreased sperm output, and abnormal sperm shape and motility (oliogoasthenoteratozoospermia). Within the seminiferous tubules of Taily males, ~26% of germ cells die at the anaphase-telophase transition of meiosis. Significant numbers of others fail to complete cytokinesis, thus resulting in multi-nucleated spermatids. Of those that do complete meiosis, all possess abnormal head and tail structures. An analysis of sub-cellular structures and confocal microscopy revealed that components of the katanin complex, including two previously uncharacterized p60-like proteins, are involved in specific microtubule-related processes in spermatogenesis. These include the shortening of the meiotic spindle during anaphase, the formation and function of the manchette and the formation of the axoneme underlying the flagella. These studies not only demonstrate the essential requirement of microtubule severing in sperm formation, but also reveal a mechanism of likely importance in other microtubule-rich tissues.

056

Capacitation-Dependent Presentation of Protein Complexes on the Surface of Human Spermatozoa

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A unique characteristic of mammalian spermatozoa is that upon ejaculation, they are unable to recognise and bind to an ovulated oocyte. These functional attributes are only realised following their ascent of the female reproductive tract whereupon the sperm undergo a myriad of biochemical and biophysical changes collectively referred to as 'capacitation'. Since spermatozoa are both transcriptionally and translationally quiescent cells, this functional transformation must be engineered by a combination of post-translational modifications and spatial reorganisation of existing sperm proteins. Indeed, evidence from our laboratory suggests that a key attribute of capacitation is the remodeling of the sperm surface architecture leading to the assembly and / or presentation of multimeric protein complexes, a subset of which have been implicated in sperm-oocyte interactions. In this study, we have extended our findings through the characterisation of one such complex comprising arylsulfatase A (ARSA), sperm adhesion molecule 1 (SPAM1) and the molecular chaperone HSPA2. Through the application of flow cytometry we demonstrated that this complex undergoes a dramatic, capacitation-associated translocation to facilitate ARSA expression on the outer leaflet of the membrane overlying the apical region of the human sperm head. Conversely, SPAM1 appears to reorient away from the sperm surface while HSPA2 remains in an intracellular location irrespective of the cells capacitation status. The dynamic reorganisation of this complex was completely abolished by incubation of capacitating spermatozoa in the presence of exogenous cholesterol, suggesting that it may be driven, in part, by alteration in the membrane fluidity characteristics. Interestingly, it was also disrupted by pharmacological inhibition of PKA, a key intermediary of the signalling machinery that drives capacitation. Collectively these findings provide novel insights into the molecular mechanisms that underpin the capacitation-associated functional transformation of human spermatozoa.

057

Does activin C promote prostate cancer malignancy?

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Prostate cancer (PCa) is a significant health issue. Broadly, two forms exist, latent organ-confined and aggressive-metastatic. Treatment options for latent PCa are available and effective, whereas treatment options for metastatic PCa are limited. Activin A is a negative growth regulator in the prostate, inhibiting cell proliferation and promoting apoptosis, favorable characteristics against PCa progression. Despite these characteristics activin A is up-regulated in PCa and via unknown mechanisms cancer cells evade activin A growth inhibition. Activin C is a novel antagonist of activin A as over-expression leads to prostatic hyperplasia in mice. Therefore it was hypothesised that activin C modulates the ability of human prostate cells to respond to activin A induced growth inhibition thus promoting a more malignant phenotype. To address our hypothesis the effect of activin A plus and minus activin C on cell growth, motility, and pathway focused gene expression (TGF-β, cell-cycle and apoptosis) was determined in human prostate cell lines PNT1A, LNCaP, DU145 and PC3, representing non-malignant, low, medium and high-grade PCa respectively. We also examined the expression of activin subunits (βA and βC) and the activin signaling molecule, Smad-2, in human prostate disease arrays. Activin C antagonized activin A mediated growth inhibition *in vitro* and increased activin-βA and βC subunit immuno-reactivity, and decreased Smad-2 were apparent in human prostate cancer. We therefore propose that local expression of activin C promotes a more malignant phenotype and therefore may possess therapeutic or diagnostic potential in human prostate disease.

058

Expression patterns of activin A and its regulatory elements in the adult male mouse reproductive tract indicate important roles for these molecules in the mature epididymis and vas deferens

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Many aspects of the biology of activin A and its binding protein, follistatin, in the male reproductive tract remain ill-defined. In the following study, expression of *Inhba* (the gene encoding the activin A subunits), *Inha* and *Inhbb* (the inhibin B subunits), follistatin (*Fst*) and follistatin-like 3 (*Fstl3*), and the activin receptor subunits (*Acvr1, Acvr2a, Acvr2b*) were measured by qRT-PCR in adult male C57Bl6 mice. A qRT-PCR assay that discriminates between the two follistatin variants, *Fst288* (the tissue-bound form) and *Fst315* (the circulating form), was established by exploiting differences in the 3'UTR of the mRNA transcripts. Activin A protein was measured by ELISA and total follistatin was measured by RIA. Activin A mRNA was most highly expressed in the epididymis, with slightly lower expression in the vas deferens and lowest expression in the testis, (ventral) prostate and seminal vesicles, in that order. Activin protein levels, however, were highest in the prostate and lowest in the testis. Activin type 1 and 2 receptor mRNA expression was highest in the corpus epididymis. Follistatin mRNA (particularly *Fst288*) and protein was highest in the vas deferens. Follistatin protein levels were also elevated in the prostate, although this was not matched by similarly elevated mRNA expression. Expression of *Inhba* and both *Fst* variants was relatively low in the testis, but inhibin α-subunit mRNA and βB-subunit was highly expressed in the testis, as expected. In contrast to the segment-specific expression of *Fst, Fstl3* was expressed at constant levels throughout the reproductive tract. The pattern of differential expression of activin A and its major regulatory elements in different segments of the male reproductive tract indicates the existence of segment-specific functions for activin A. In particular, these data point towards an important role for activin A and follistatin in regulating the different environments of the epididymis and vas deferens.

059

Copy Number Variation In Testis Cancer

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Type 2 testis tumours represent the most common cancer in adolescents and young men, with a median diagnosis age of approximately 30 years. Little is known of the genetic pathways that underlie formation and progression of testis cancer, and it is considered that the development of more advanced testis cancer biomarkers is key to enhancing the understanding of testis tumour biology. Testis tumour cells have genomic imprinting patterns, telomerase activity and gene expression profiles similar to those observed in fetal germ cells, strongly indicating that testis cancers originate from fetal germ cells that have failed to mature. Previously, we have identified genes which are ≥four fold differentially expressed during mouse male fetal germ cell development. We have used multiplex ligation-dependent probe amplification to screen several of these genes for duplications or deletions in 250 testis cancer patient samples (125 seminoma and 125 non seminoma). Of the genes that show aberrant copy number variation in these samples, three have previously been implicated in cancer aetiology. This analysis has identified copy number variation in testis cancer tumour samples in genes that are regulated during male fetal germ cell development.

060

Ovarian Extracellular Matrix and in Health and Disease.

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Follicles and corpora lutea continually remodel during their development and regression and whilst hormones and growth factors are key regulators of this so too is extracellular matrix. Cells bind directly to matrix and the composition and rigidity of matrix influence cell behaviour. Different matrix molecules can also bind growth factors or their binding proteins as a means of limiting the spread and hence the bioavailability of growth factors within tissues. Therefore our laboratory has focused on matrix in ovaries, first characterising its composition and latterly determining its roles. We found that the follicular basal lamina dynamically changes in composition during follicular development and it is degraded at ovulation and new matrix is produced during corpus luteum formation (1,2). The follicular basal lamina is not degraded as part of atresia but can be breached by

macrophages (1,2). Some follicles also have additional layers of follicular basal lamina, similar to diabetic organs, and these follicles have poorer quality oocytes (3). A novel matrix is also produced between the granulosa cells, focimatrix (4), and we believe it is involved in inducing steroidogenesis and follicle dominance (5,6). The formation of follicular fluid maybe due to the osmotic pressure and large sizes of versican and hyaluronan (7,8). Genetic linkage studies have identified a region of the genome linked to polycystic ovary syndrome (PCOS), and this regions is flanked by the gene for fibrillin-3, and fibrillins bind latent TGFβ binding proteins. As such they control the bioavailability of TGFβ and TGFβs stimulate collagen production and fibrosis. The PCOS ovarian phenotype has the hall marks of a fibrosis and PCOS may have a fetal origin. Linking these facts is our observation that fibrillin 3 is expressed in fetal ovaries when the stroma is expanding and follicles are forming (9). We conclude that matrix is important in ovaries.

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061

Uterus transplantation – Animal research towards clinical introduction

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The last frontier in efforts to treat female infertility is absolute uterine infertility. The major groups of women with uterine fertility are those that lack a uterus from birth (MRKH-syndrome) or through hysterectomy (cervical cancer, myoma, emergency peripartum hysterectomy) in addition to those that have a defect uterus (severe intrauterine adhesions, large leiomyoma, malformation). Uterus transplantation (UTx) is a possible future treatment for these infertile women.

Our research group has developed several animal UTx models (mouse, rat, sheep, baboon) that we have used during the last decade to develop the UTx- procedure towards introduction in the human.

In the mouse model, we achieved syngeneic uterine transplants by end-to-side aorta-aorta/vena cava-vena cava vascular anastomoses, with later proven fertility after 24-h cold ischemia and transplantation. Live birth from a transplanted uterus was first demonstrated in the mouse model, in 2003. In allogeneic transplants of the mouse uterus, we characterized inflammatory-cell influx at uterine rejection, showing an early invasion of T-cells with later accumulation of macrophages. Monotherapy with cyclosporine decreased rejection-related inflammation in the allogenic mouse UTx model. In our allogenic rat UTx model, monotherapy with low-doses of either tacrolimus or cyclosporine prevented rejection and fertility has for the first time been demonstrated in an allogenic UTx model. These offspring were of normal birth-weight and developed normally during an observation time of > 8 months. These results show for the first time that pregnancy can be established and grow normal in a transplanted uterus with new vascular connections and under influence of immunosuppressants to prevent rejection.

Our UTx research has also come to include the larger sheep model. Surgical techniques in the sheep auto-UTx model included vascular anastomosis end-to-side to the external iliac vessels. Fertility was demonstrated after spontaneous mating with live-offspring after caesarean section at full-term gestation.

During the last 3 years we have also utilized the non-human primate baboon UTx model. In initial experiments with auto-UTx, the graft was retransplanted back after 3 h of cold ischemia. Vascular connections of the uterine vessels were established end-to-side to the external iliac vessels. The long-term success in terms of resumed menstruation was only 20% in the initial series of ten animals but has improved to 60% in the second series. Importantly, the survival rate of the animals was 100%. Allogenic UTx in the baboon has also been conducted with demonstration of 100% survival of donors and recipient animals. The transplanted uterus showed rejection when only monotherapy with tacrolimus was used but later attempts with inclusion also of induction therapy (anti-thymocyte globulin) and steroids have demonstrated long-term survival in animals. Ongoing studies are directed towards comparing different vascular anastomosis sites and the living-donor/deceased donor concept in the allogenic baboon UTx-model.

Studies in the human have shown ischemic tolerance of human uterine tissue for 24 h and also that reasonable (> 6cm) lengths of the uterine arteries/veins can be harvested at hysterectomy and this will allow for bilateral anastomosis to the external iliac vessels in a human UTx situation with an organ from a living donor.

In conclusion, the research towards human UTx is progressing well and it is anticipated that human transplantation trials will be performed within the next few years.

062

Decidual Cell Surface and Secreted Factors Regulate Trophoblast Function

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Extravillous trophoblast (EVT) must adhere, migrate and invade through the decidua during the formation of a functional placenta. Abnormal decidualization of endometrial stromal cells (ESC) leads to unregulated trophoblast invasion and pregnancy failure in mice. Recent evidence in humans suggests that preeclampsia is associated with impaired decidualization. The mechanisms by which decidual cells interact with EVT remain largely unknown. We aimed to determine whether decidualized and non-decidualized human (H) ESC regulate EVT adhesion and outgrowth.

Primary HESC were decidualized with cAMP + estradiol 17β+ medroxyprogesterone acetate and prolactin (decidual marker) measured by ELISA. HESC conditioned media (CM) was collected from Days (D) 0-2 (non-decidualized) and D12-14 (decidualized) of treatment to allow the treatments to be equally processed by the cells. Cytotrophoblast were isolated from 1st trimester placenta and cultured on Matrigel^{TM} for EVT. Villous tips were seeded onto collagen drops for 48h and cytotrophoblast outgrowth measured. Proteins <30kD were purified from non-decidualized and decidualized CM using size exclusion affinity nano-particles (SEAN) and identified by mass spectrometry.

Fluorescently labelled EVTs showed increased adhesion to decidualized compared to non-decidualized HESC (184.5±45% vs 100% respectively, n=5; p<0.05). Decidualized CM enhanced cytotrophoblast outgrowth from villous tips isolated from week 9 of gestation (100 vs 144%, n=5) however decidualized CM impaired outgrowth in villous tips isolated from weeks 7, 10 & 12 of gestation (100 vs 42%, n=4). Proteomics identified 7 unique proteins secreted by decidualized CM: 3/7 not previously been identified in the decidua and 2/4 known proteins are associated with placental pathologies.

This study demonstrates that decidualized/non-decidualized secreted factors differentially regulated EVT function and further, for the first time demonstrated that trophoblasts from different weeks of gestation responded differently to decidual cues. In conclusion, synchronized decidualization and trophoblast maturity may be critical for appropriate EVT function and therefore placental development in humans.

063

Epigenetic Mechanisms Regulating Prostaglandin Endoperoxide Synthase-2 (PTGS2) Expression in the Amnion During Pregnancy.

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PTGS2 expression increases in the amnion with advancing gestation, contributing to the production of prostaglandins that stimulate labor. The mechanism regulating this increase is unknown; however, epigenetic chromatin modifications have been implicated in programming tissue specific patterns of gene expression during development. Epigenetic programming of PTGS2 expression was examined by measuring the levels of activating and repressive histone modifications located at the PTGS2 gene during pregnancy and correlating the histone marks with PTGS2 expression.

Amnion was collected early in pregnancy (16-18 weeks) , at elective caesarean section and following spontaneous labour at term. Chromatin immunoprecipitation (ChIP) was used to determine the levels of the activating epigenetic modifications histone-3 lysine-4 trimethylation (H3K4me3), histone-3 lysine-36 trimethylation (H3K36me3), histone-3 acetylation (acH3) and histone-4 acetylation (acH4) and the repressive epigenetic modifications histone-3 lysine-27 trimethylation (H3K27me3) and histone-3 lysine-9 trimethylation (H3K9me3) at the PTGS2 gene.

The active histone marks acH3, acH4 and H3K4me3 accumulated in the promoter region close to the transcriptional start site and levels increased as gestation advanced. H3K27me3 spanned the promoter and transcribed region of the gene with levels decreasing around the TATA site. H3K27me3 levels were high early in gestation and decreased at term. H3K36me3 levels peaked in the 3' region of the gene. H3K9me3 was present on the gene.

Active and repressive histone modifications mark the PTGS2 gene. The changes with advancing gestation are consistent with a switch from a repressive to a permissive chromatin structure, suggesting that the PGHS2 gene is activated by epigenetic mechanisms at term allowing increased expression and prostaglandin production to promote the onset of labour.

064

Fibroblast activation markers in ectopic and eutopic endometrium from women with endometriosis

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Endometriosis is a disease characterised by inflammation, extensive fibrosis and tissue scarring, all processes involving fibroblast activation. Our aim was to examine the activation status of stromal fibroblasts in eutopic versus ectopic endometrium from women with endometriosis. Endometriotic lesions and eutopic endometrium were collected from women undergoing laparoscopy for the treatment of endometriosis (n=4 proliferative, n=7 secretory). Samples were screen for endometrial glands and immunostained for CD10 to identify endometrial stroma. Laser capture microscopy was used on serial sections to collect glandular epithelium and CD10-postive stroma from ectopic lesions and eutopic endometrium, as well as CD10-negative stroma from ectopic lesions. Quantitative PCR was used to examine the mRNA expression of selected genes involved in fibroblast activation (*TGFβ1*, *NFκB1*, *ACTA2*, *IFNγ*, *SMAD2*, *SMAD3* and *SMAD4*). mRNA was expressed relative to an RNA spike. *18S* rRNA expression was also examined; however, expression varied between eutopic and ectopic sites making it an unsuitable housekeeping gene in this model. Expression of *TGFβ1*, *SMAD2*, *SMAD3* and *SMAD4* mRNA was significantly higher in eutopic versus ectopic endometrial glandular epithelium. However, there was no significant difference in the expression of these genes in eutopic stroma, ectopic CD10-positive stroma or ectopic CD10-negative stroma. There was no significant variation in *ACTA2*, *NFκB1*, or *IFNγ* mRNA expression among any of regions examined. Variations in *TGFβ1*, *SMAD2*, *SMAD3* and *SMAD4* mRNA expression in eutopic relative to ectopic glandular epithelium are consistent with a specific function for the TGFβ pathway in eutopic endometrium that is not replicated in ectopic lesions. However, our data do not support a difference in fibroblast activation status between CD10-positive endometrial stromal fibroblasts in ectopic lesions versus eutopic endometrium or tissues adjacent to CD10-positive lesions. Further studies examining the protein expression of key fibroblast activation markers and *in vitro* studies with isolated endometrial cells are planned.

065

Antiphospholipid Antibodies and Trophoblast Shedding: A Real Phenomenon Associated With Mitochondrial Interactions

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Introduction: Antiphospholipid antibodies (aPL) are a predisposing factor for a common disease of pregnancy, preeclampsia. Preeclampsia is triggered by placental factors, one of which may be trophoblastic debris, which is shed from the placental syncytiotrophoblast. We have previously shown in vitro that aPL alter the nature and amount of shed trophoblastic debris, possibly explaining why preeclampsia is so common in women with aPL. However, the suitability of this in vitro model has been questioned since some argue that after culture, artifactual delamination and shedding of the trophoblast can be observed with electron microscopy. In addition to the model's potential shortcomings, it is not actually known how aPL affect trophoblast shedding.

Methods: First trimester placental explants were cultured for 48 hours then stained with the vital stains Cell Tracker Green and propidium iodide and visualised by confocal microscopy. These explants were then fixed immediately with glutaraldehyde and sections examined by light or transmission electron microscopy (TEM). Separate placental explants were incubated with gold-labelled murine monoclonal aPL then fixed with glutaraldehyde, sectioned then examined by TEM.

Results: Investigation of live placental explants revealed that after 48 hours of culture, the trophoblast did not delaminate. In contrast, following glutaraldehyde fixation, gaps with a mean size of 8.6 μ m were present between the trophoblast and the villous stroma. Gold-labelled aPL were associated with syncytiotrophoblast mitochondria.

Conclusions: That trophoblast delamination was observed only after fixation - and not in the same explants prior to fixation - suggests that delamination is a fixation artefact unrelated to culture. This validates our in vitro model for studying the effects of aPL on the placenta. The localisation of aPL with mitochondria underscores the potential of these autoantibodies to affect the syncytiotrophoblast death process, thus affecting the shedding of trophoblast debris and the development of preeclampsia.

066

Why it is essential to regulate mitochondrial DNA replication during oogenesis and embryogenesis.

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During oogenesis and preimplantation development, mitochondrial DNA (mtDNA) replication is strictly regulated so that, by the blastocyst stage, the inner cell mass cells, which have the potential to give rise to all cell types of the body, possess fewer copies of mtDNA than the trophectodermal cells. The inner cell mass cells also give rise to embryonic stem cells and, in their undifferentiated state, they possess significantly fewer copies and maintain immature mitochondria. This continual reduction results in the establishment of the 'mtDNA-set point'. From this set point, all pluripotent embryonic stem cells can acquire the appropriate numbers of mtDNA to match their specific requirements for OXPHOS-derived ATP once they have fully differentiated. As embryonic stem cells differentiate, there are several distinct mtDNA replication events, which are driven by the nuclear-encoded mtDNA-specific replication factors. However, one replication event is critical to the continued survival of the cell whilst another is associated with the accumulation of mtDNA mass later during differentiation. Nevertheless, reprogrammed somatic cells generated through somatic cell nuclear transfer, cell fusion techniques and induced pluripotency do not appear to regulate mtDNA replication in a similar manner. They have significantly higher numbers of mtDNA copies in their pluripotent state or are primed to trigger premature mtDNA replication. During differentiation, they fail to achieve the two key mtDNA replication events observed in embryonic stem cells and have aberrant patterns of expression for the nuclear-encoded replication factors. Nevertheless, by culturing induced pluripotent stem cells with inhibitors of de novo DNA methylation, which act on the nuclear-encoded mtDNA replication factors, we have been able to mimic the mtDNA replication events of embryonic stem cells. These outcomes demonstrate that the regulation of mtDNA replication during differentiation and development is dependent on the epigenetic control of the nuclear-encoded mtDNA replication factors.

067

The fine 'ART' of endometrial receptivity

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Aim: Receptivity of the endometrium in assisted reproduction (ART) cycles is an important consideration. Immunohistochemical, genomic and proteomic studies demonstrate that the ART endometrium is altered versus natural-cycles. A recent study examining frozen embryo transfers demonstrated significantly lower ongoing pregnancy rates in women who received hCG vs transfers following the natural LH-surge (1). We therefore investigated whether hormones administered during ART cycles, particularly hCG, cause the endometrium to become refractory to blastocyst signals at implantation.

Methods: Immunohistochemistry with semi-quantitative scoring determined localization/expression of the LH/CG receptor (R) in cycling endometrium (n=10 per cycle stage) compared with endometrium from ART cycles at hCG+2 (GnRH antagonist not pregnant (np) n=10, GnRH agonist (np) n=16, GnRH agonist pregnant n=12). In vitro experiments aimed to mimic ART cycle exposure to hCG. These establish the effects of pre-exposure to a low dose of hCG, prior to a 'high dose' of hCG (20IU) mimicking blastocyst hCG secretion. LHCGR expression, activation of signaling (Western immunoblot), epithelial tight junction integrity (transepithelial resistance) and cell adhesion (adhesion assay) were determined in the HES cell line in response to treatment with 0.5 - 5IU hCG for 3-5 days followed by 20IU hCG.

Results: Expression of LHCGR was decreased in glandular and luminal epithelium in the agonist np group vs early/mid secretory endometrium ($p<0.05$). In cells pre-exposed to hCG the LHCGR was down-regulated and re-localized and ERK phosphorylation was reduced in response to 20IU hCG. Epithelial tight junction integrity could not be 'relaxed' and adhesion of endometrial epithelial cells to blastocyst-expressed fibronectin or collagen I or IV was reduced upon pre-exposure.

Conclusions: Exposure to hormonal regimens in ART cycles decreases LHCGR expression. Prior exposure specifically to hCG causes alterations in receptor expression and localization with the endometrial epithelium becoming refractory to blastocyst signals as indicated by physiologically-relevant assays.

(1) Fatemi et al. Fertil Steril. 2010. 94(6):2054-8.

068

The identification and functional characterisation of mutant isoforms of the oocyte activation factor phospholipase C zeta (PLCζ), and their genetic modes of inheritance.

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Mammalian oocytes are thought to be activated by a sperm-specific phospholipase C, PLCzeta (PLCζ). Molecular analysis of genomic DNA from infertile patients focusing on each PLCzeta exon, and associated flanking sequences, successfully identified a number of changes to the PLCzeta gene sequence. In one case, analysis identified a point mutation in the PLCzeta gene in an infertile male exhibiting normal sperm morphology, but defective oocyte activation capability, leading to a significant change in a conserved Histidine at position 398 in the catalytic region of the protein to a Proline (H398P), that may inhibit normal function. Here, we describe a novel mutation in the PLCzeta gene, identified from the same patient from whom H398P was isolated, and also occurring in the PLCzeta protein's active site. We have functionally characterised this new mutation in both mammalian cells and oocytes utilising fluorescence and confocal microscopy and calcium imaging, and have also investigated the modes of inheritance of both mutations. We show that both the novel mutation and H398P are heterozygous mutations, both occurring on separate alleles, with H398P inherited from the patient's father. We also show for the first time the inheritance of a male factor infertility condition (the novel mutation) passed on by the patient's mother to her son. Upon further investigation, we show the inheritance of this mutation in the patient's daughter, conceived via assisted reproductive techniques (ART). Our studies indicate that defective forms and expression levels of PLCζ may play an important role in certain types of male factor infertility, and suggests that an active recombinant PLCζ may represent a valuable therapeutic tool for such patients. Results also indicate that the modes of inheritance of male factor infertility conditions are not contained to paternal modes, but may also be linked to maternal modes of inheritance, and that ART interventions could potentially transfer fertility problems from parent to progeny.

069

Pluripotent Stem Cells From The Testis

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Recent reports have demonstrated that adult cells can be reprogrammed to pluripotency, but mostly with genes delivered using retroviruses. Some of the re-programming genes and the retrovirus used to deliver them are tumorigenic, thus, these adult-derived embryonic stem (ES)-like cells cannot be used to cure human diseases. Remarkably, it has also been demonstrated recently by several groups that, in mice and humans, spermatogonial stem cells(SSCs) can be reprogrammed to ES-like cells without the necessity of exogenously added genes. The *Nanog* homeobox gene plays a central role in the molecular regulation of pluripotency in the embryo and in embryonic stem cells. Here, we demonstrate using genetically modified mice, carrying a LacZ reporter inserted into the Nanog gene, that pluripotent stem cell lines can be isolated from the adult mouse testis. Pluripotent stem cell lines were established without gene transduction and maintained using well established tissue culture protocols. This approach can potentially be used to generate stem cell lines for personal stem cell medicine, analysis of genetic disease, improvement of livestock and preservation of rare or endangered species.

070

Generation of Wild Cats iPS cells, using Nanog as a additional factor in the reprogramming cocktail.

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Since the first report on the generation of induced pluripotent stem cells from mice in 2006, the approach has been explored in various species as it provides a unique approach to understanding pluripotency and lineage commitment in species where embryonic stem cells (ESCs) have been difficult to isolate. This applies more so for endangered species where the limited availability of gametes makes research on ESCs unfeasible. Induction of pluripotency from wild cats, including from Bengal tiger, Jaguar and Serval (TiPS, JiPS, SiPS) was attempted on dermal fibroblasts by retroviral transfection with genes encoding five human transcription factors; Oct-4, Sox-2, Klf-4, cMyc and Nanog. Transduction efficiency estimated using a GFP reporter construct ranged from 93-99%. Infection with the reprogramming cocktail resulted in the formation of colonies, which were able to be maintained in culture and exhibited hall marks of ESCs. TiPS, JiPS and SiPS colonies which formed as early as Day 3, were picked at day 5 and expanded *in vitro* on feeder layers. The resulting colonies from the three species were positive for alkaline phosphatase (AP), Oct-4, Nanog and SSEA-4, at P14. RT-PCR confirmed that all 5 human transgenes were transcribed at P4 but Oct-4, Sox-2 and Nanog transgenes were silenced as early as P14, suggesting reprogramming and reactivation of the endogenous pluripotency genes. Endogenous Oct-4 and Nanog were first expressed by the wild cats iPS cell from P4. When injected in Severe Combined Immune Deficient (SCID) mice, TiPS, JiPS and SiPS cells at P18 formed teratomas containing a variety of tissues representative of the three germ layers. This study describes robust methods for inducing pluripotency in a range of endangered wild cats (i.e.) Bengal tiger, Jaguar and Serval and provides insights on pluripotency in felid species. These cells provide a unique source of pluripotent cells with utility in conservation for cryopreservation of genetics, as a source of reprogrammed donor cells for nuclear transfer or for directed differentiation to gametes in the future.

071

On the Survival and Development of the Preimplantation Embryo

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The development of the preimplantation embryo is a seemingly autopoietic process. This may in part be explained by the actions of autocrine tropic ligands. Their action is illustrated by the developmental benefits bestowed by culture of embryos in groups in vitro, and characterization of their mode of action is well advanced.

The actions of autocrine ligands are required by the mouse 2-cell stage of development, and they can act in concert with paracrine mediators present within the reproductive tract. Their activation of the 1-o-phosphatidylinositol-3-kinase signaling pathway is essential for normal development. This pathway has the minimum dual roles of activating calcium/calmodulin-dependent kinase activation of the CREB transcription factor and AKT (protein kinase B)-mediated suppression of the action of the tumour suppressor, P53.

Tropic ligands, such as Paf, cause the activation of CREB at the time of the activation of transcription from the embryonic gene. CREB drives transcription of pro-survival effectors, including the proto-oncogenes c-FOS and BCL2. AKT induces the phosphorylation and activation of MDM2, and this causes the ubiquitination and resultant degradation of P53. Reduced autocrine signaling is caused by a range of stresses (including the production and culture of embryos in vitro), and this results in reduced expression of key proto-oncogenes and the up-regulation of P53 activity. P53 reduces the rate of cell proliferation and increases apoptotic cell death, particularly within the inner cell mass.

Tropic signals provide coordinated mechanisms for maintaining the survival of the cells of the early embryo. Disturbance of survival signaling has the net effects of reducing the number of cells populating the early embryo and reducing the pluripotency of the remaining cells. The resultant embryos have a markedly reduced capacity for implantation and those that do implant are likely to be abembryo.

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