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AWARDS

Novartis Pharmaceuticals

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Serono

Research Centre for Reproductive Health

Reproduction, Fertility and Development
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### SRB OFFICE BEARERS 2006

**President**  Lois Salamonsen  
**Deputy President**  Sarah Robertson  
**Secretary**  Caroline Gargett  
**Treasurer**  Kate Loveland  
**Committee**  Marie Pantaleon, Darryl Russell, Chris Scott, Jean Fleming  
**Communication Officer**  Melinda Jasper  
**Postgraduate Representatives**  Tu‘Uhvehava Kaitu‘U, Janette Quennell

### PAST SRB OFFICE BEARERS AND AWARDEES

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The Local Organising Committee
SRB: Grant Montgomery (Chair), Jean Fleming, Marie Pantaleon
ESA: Judith Clements

SRB Program Organising Committee
Sarah Robertson, Eileen McLaughlin, Darryl Russell, Ann Drummond, Sarah Meacham & Claire Roberts

ESA Program Organising Committee
Stephen Twigg (Chair), Anne Nelson, Charles Allan, Roderick Clifton-Bligh, Shaun McGrath & Paul Williams

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

FUTURE MEETINGS

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SRB Founders Lecturer

Marilyn Renfree
Marilyn's primary research interest is the developmental biology, reproductive physiology and endocrinology of mammals. Her research has been almost all on marsupials, because their unique reproductive strategy provides unrivalled opportunities to understand these processes. Marsupials give birth to small underdeveloped young that undergo the majority of their development external to their mother in the pouch, making it possible to study the control of normal organ growth throughout developmental stages otherwise inaccessible in utero. Her laboratory is known internationally for its innovative studies of these unique and charismatic Australian animals. Marilyn is a Laureate Professor of the University of Melbourne and an ARC Federation Fellow.

Meat and Livestock Plenary Lecturer

Bill Thatcher
William W. Thatcher is a Graduate Research Professor Emeritus in the Department of Animal Sciences at the University of Florida. He received his B.S. from the University of Maryland, a M.S. degree from the University of Maryland, in conjunction with the USDA-ARS Beltsville Research Center, and the Ph.D. degree from Michigan State University. He completed two sabbaticals at INRA, Nouzilly, France, in 1977 and 1985. His research program in cattle has been associated with ovarian follicular development, maternal-embryo interactions, and developmental approaches for regulating reproductive function to enhance production and health. He has served as a mentor for 70 graduate students-postdoctoral fellows and sabbatical persons. Dr. Thatcher has published 311 refereed journal articles and 40 book chapters (http://www.thatcherteam.com).

Other Plenary Speakers

Eiichi Araki
Professor Araki is Professor and Director of the Department of Metabolic Medicine, Faculty of Medical and Pharmaceutical Sciences, Kumamoto University. His honours and awards include the 1989 Kato Memorial Young Investigator's Research Award, 1990 Young Investigator's Award of the Japanese Society of Internal Medicine, 1994 Mary K. Iacocca Research Fellow (honorary fellow), 1998 Shionogi-Lilly Award, Japan Diabetes Society and the 1999 Young Investigator's Award, Kumamoto Medical Society.

William Crowley M.D.
Dr. William Crowley is a Professor of Medicine at Harvard Medical School, Director of the Harvard Reproductive Endocrine Sciences Center, Chief of the Reproductive Endocrine Unit and Director of Clinical Research at the Massachusetts General Hospital (MGH). He graduated from Holy Cross College in 1965, Tufts Medical School in 1969, and has been at the MGH since 1969. Dr. Crowley is an active clinical investigator whose main research interest is discovering the genes that control reproduction in the human using various disease models of both sexes. He also directs a Training Program for clinical and basic investigators in Developmental Biology and Reproductive Endocrinology and has an active interest in the national program of clinical research in academic health science centers. He is the Founder and current President of the Clinical Research Forum, an association of 48 of the US's leading academic medical centers involved in human research. He is also the past President of the Endocrine Society, the recipient of the 2005 Fred Conrad Koch Award (The Endocrine Society's highest scientific award), The Mentor of the Year Award from Women in Endocrinology (first male recipient), a former Board Member and Executive Committee member of FASEB, and a former member of the Institute of Medicine's Clinical Research Roundtable.

Creswell J Eastman
Endocrinologist and former President of Endocrine Society of Australia, Vice President Asia Oceania Thyroid Association, Chairman of ACCIDD and Vice Chairman of Board of International Council for Control of Iodine Deficiency Disorders (ICCIDDD)

Sadaf Farooqi
Dr I. Sadaf Farooqi qualified with honours in Medicine from the University of Birmingham in 1993 and was awarded the Queen's Scholarship (gold medal) for best overall academic performance. After junior hospital posts in Birmingham and Oxford she moved to Addenbrooke's Hospital, Cambridge, as part of a Wellcome Trust Training Fellowship. During this time of her PhD, she identified the first single gene defect to cause human obesity in two children with a mutation in the gene encoding the hormone leptin, published in Nature in
1997 and New England Journal of Medicine in 1999. Since the award of a Wellcome Trust Clinician Scientist Fellowship in 2002, Sadaf has continued studying the genetic basis of severe human obesity at the Dept of Clinical Biochemistry, Cambridge, in parallel with her clinical training in General Medicine, Diabetes and Endocrinology. She has been invited to speak at numerous International research meetings and has recently described mutations in other genes such as MC4, WNT10B and TrkB, which may have functional consequences for cases of human obesity.

**Peter Leedman**

Peter Leedman graduated in medicine (UWA) and completed training in endocrinology and his PhD (WEHI) before a post-doc at Harvard Medical School with Bill Chin. He returned to Perth in 1994 to take up an academic position in medicine at UWA based at Royal Perth Hospital. Since then, he has been recognized internationally for his work on hormone action, in particular the mechanisms regulating expression of key targets for therapeutics in hormone-dependent cancer (including the androgen and growth factor receptors). More recently, the discovery of several novel nuclear receptor coregulators that modulate estrogen and androgen signaling, has provided a range of new potential biomarkers and therapeutic targets. He has received several awards, including the UK Endocrine Society Asian and Oceanic Medallist in 2004. He currently serves on the editorial boards of the Journal of Molecular Endocrinology and Endocrine Reviews and is Deputy Director of the Western Australian Institute for Medical Research (WAIMR) in Perth.

**Manuel Tena-Sempere**

M. Tena-Sempere is presently holding a position as Associate Professor in Physiology at the University of Cordoba, Spain. His expertise lies within the field of Reproductive Biology and Neuroendocrinology. His main areas of interest within Reproductive Endocrinology include: (1) Analysis of the neuroendocrine networks controlling gonadotropin secretion, with special attention to kisspeptin physiology; (2) Characterization of the molecular mechanisms and physiological signals responsible for the integrated control of reproduction, energy balance and metabolism; (3) Identification of novel markers and molecular mechanisms for endocrine disruption of the reproductive axis at the hypothalamic-pituitary level; and (4) Study of gonadal physiology, with special attention to the testis. In the last 12 years, M. Tena-Sempere has published over 100 articles in international peer-reviewed journals on the topics indicated above.
STUDENT MEMBERS

Student Members

There are four events at the 2006 SRB Annual Conference that students should not miss!

Sun 20th Aug 2pm  SRB Workshop “Talking science to the media”  M5&M6
Sam Elam (Media Manoeuvres) and Sarah Meacham (Chair)

Mon 21st Aug 6pm  SRB Students Annual Meeting  M6
This is a great opportunity to meet fellow students working in the field of reproductive biology, and voice your opinions on how we can improve your experience of SRB student membership. SRB travel awards are also given at this meeting.

Mon 21st Aug 7pm  Student Supervisor Mixer  BOJANGLES, Broadbeach
Don't miss this chance to meet and have a great night with other SRB and ESA students, and network with other conference delegates working in your field.

Tues 22nd Aug 12pm  Student lunch with Founder’s Lecturer  M5
*Founders’ Lecturer, Marilyn Renfree, will be participating in a relaxed roundtable discussion specifically for students. All students are welcome to attend. Collect your lunch and join in.*

WANT TO BECOME AN SRB STUDENT MEMBER?
For only $35 per year, membership gives you:
Discount registration at SRB Annual Scientific Meetings
Free SRB Abstract Booklets and Newsletters
Eligibility for SRB Travel Awards and the New Investigator Award
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 Affiliate Membership of SSR
Please see your Australian Postgraduate Student Representative, Tu'uhe Kaitu'u-Lino.
Email: Tuuhevaha.kaituu@princehenrys.org
INFORMATION FOR DELEGATES & PRESENTERS

Venue Location
Gold Coast Convention and Exhibition Centre
Cnr Gold Coast Highway & TE Peters Drive
Broadbeach, Queensland 4218
Phone: +61 7 5504 4000
Fax: +61 7 5504 4001
The Gold Coast Convention and Exhibition Centre (GCCEC) is located in Broadbeach, on Australia’s Gold Coast in Queensland. It is a unique destination which caters for both leisure and business tourism. Close to a variety of accommodation, shopping, restaurants, nightlife, entertainment and 70 kms of pristine white sandy beaches.

Session Locations
The Conference activities are spread out over two levels. Please refer to map below.

Organiser’s Office and Registration Desk
The organiser’s office and registration desk will be located on ground floor, main entrance of the Gold Coast Convention Centre. The office and desk will be attended at all times during the conference from 7:30am in the morning. Delegates should collect their satchel, name tag and other conference material on arrival. A message board will be placed immediately inside the main Exhibition entrance.
The Speaker Preparation Room
The speaker preparation room is accessed through the organiser’s office. Networked computers in this room will allow MS PowerPoint presentations loaded here to be shown in any of the session rooms. Technicians and assistants will be in attendance in the room and speakers are encouraged to load their presentations as soon as possible to avoid any last minute rushes.

Registration
Conference delegates receive the following services as part of their registration:
- Access to all scientific sessions on day(s) of registration
- A satchel with a copy of the delegate handbook and abstracts*
- Lunches on Monday, Tuesday and Wednesday
- Morning teas on Monday, Tuesday and Wednesday
- Afternoon teas on Monday and Tuesday
- The Welcome Function on Sunday evening

*All delegates receive a copy of the proceedings, but satchels can only be given to trade delegates if supply allows

Name Tags
Delegates are required to wear their name tags to all scientific and catered sessions.

Social Functions
- The Welcome Function is in the Gold Coast Convention Centre on the Sunday evening from 6pm. Light refreshments and drinks will be served and the function is complimentary for all registration types.
- The Women in Endocrinology Function will follow the Welcome Function at 7pm. Again light refreshments and drinks will be served. This is a ticketed function and they must be purchased in advance.
- The Monday night Student Function is being held at Bojangles, Broadbeach (across the road from Gold Coast Convention Cente). Those who have already purchased a ticket should find their ticket with their registration papers on arrival. The ticket cost includes your meal, entertainment and drinks for the first three hours. The function begins at 7:30pm and dress is neat casual. This is a ticketed function and they must be purchased before the night.
- The Conference Dinner will be held onsite at the Gold Coast Convention Centre, first level, Foyer E. Pre-dinner drinks will be served from 7:00pm for a 7:30pm start. Dress is neat casual. Entertainment for the night is 'Don't Fret'. This is a ticketed function and they must be purchased in advance.

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.

Delegate Passport Competition - ASN is again sponsoring a delegate passport competition. Your entry form is in your satchel. Place completed entries in the competition box at the registration desk by 5pm Tuesday.

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.

Occasional Meetings - A number of special meetings and functions have been called by various interested parties throughout the conference. Those involved and uncertain of which room they should be in will be able to obtain guidance from 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

Sunday, 20 August 2006

SRB Workshop - Talking Science to the Media
2:00 PM - 3:30 PM
Chair: Sarah Meachem
Presenter: Sam Elan, Media Manouvres

Afternoon Tea
3:30 PM - 4:00 PM
1st Floor Foyer

SRB Symposium - Where Genotype Meets Phenotype; The New Frontier
4:00 PM - 6:00 PM
Co-Chairs: Grant Montgomery and Jean Fleming

4:00pm  John Mattick  The role of non-coding RNA in human development  abs#001
4:50pm  Peter Koopman  Why men make sperm and women make oocytes: Discovery of the molecular signals controlling
germ cell fate during embryonic development  abs#002
5:25pm  Gail Risbridger  A novel approach to study human prostate development and its diseases  abs#003

ESA / SRB Welcome Function
6:00 PM - 7:30 PM
1st Floor Foyer

ESA / SRB Women in Endocrinology Function
7:00 PM - 8:00 PM
M4  Session sponsored by DSL
Monday, 21 August 2006

ESA Taft Plenary
8:30 AM - 9:30 AM
Chair: Jeffrey Zajac

Sadaf Farooqi
Lessons from Monogenic Obesity Syndromes  abs#004

SRB Orals - Male Reproductive Tract
8:30 AM - 9:45 AM
Co-Chairs: Richard Ivell and Moira O’Bryan

8:30am  Pradeep Tanwar
Bone morphogenetic protein-4 immunolocalization is developmentally regulated in mice testis  abs#201

8:40am  Laura Parry
Relaxin deficiency does not result in developmental abnormalities in the prostate gland of adult mice  abs#202

8:50am  Marilyn Renfree
The effects of oestrogen on mammalian sexual determination  abs#203

9:00am  Hongshi Yu
Characterization and Expression Patterns of WNT4 During Gonadal Development in the Marsupial, Macropus Eugenii  abs#204

9:10am  Jennifer Scott
Presence of TGF-β but not IL-8 or GM-CSF in ram seminal plasma  abs#205

9:20am  Nanette Schneider
The sense of smell in the reproduction of the tammar wallaby (Macropus eugenii)  abs#206

9:30am  Phillip Matson
The effect of poly-l-lysine and urine volume upon the adhesion of numbat and dibbler sperm to microscope slides.  abs#207

SRB Orals - Placental Development and Function
8:30 AM - 9:45 AM
Co-Chairs: Neil Gude and Amanda Sferruzzi-Perri

8:30am  Kirsty Pringle
Oxygen, insulin-like growth factor-II (IGF-II) and their interactions in murine trophoblasts in vitro  abs#208

8:40am  Joanna James
The effects of oxygen concentration and gestational age on extravillous trophoblast outgrowth from first trimester villous explants  abs#209

8:50am  Eleanor Ager
The function and evolutionary significance of genomic imprinting in the marsupial placenta  abs#210

9:00am  Neil Gude
Proteomic analysis of human placenta identified increased expression of chloride intracellular channel 3 with pre-eclampsia  abs#211

9:10am  Lloyd White
Caspase-14: a new player in cytotrophoblast differentiation  abs#212

9:20am  Kaori Koga
Cyclic AMP stimulates soluble vascular endothelial growth factor receptor 1 (sVEGFR-1) production in human cytotrophoblast.  abs#213

9:30am  Gayathri Rajaraman
Opposing effects of HGF and TGF-β on HLX1 expression in human trophoblast cells  abs#214
ESA Servier Award
9:30 AM - 9:45 AM  
Arena 1B
Chair: Gail Risbridger
The 2006 Servier Award winner is Renea Taylor
Formation of Human Prostate Tissue from Embryonic Stem Cells

Morning Tea
9:45 AM - 10:15 AM  
Exhibition Hall

ESA Novartis Junior Investigator Award Finalists
10:15 AM - 11:45 AM  
Arena 1B
Co-Chairs: Jeffrey Zajac and Amy Au  
Session sponsored by Novartis

10:15am  
David MacIntyre
Evidence for a novel mechanism for human myometrial activation  abs#101

10:30am  
Patrick Lim
The classical androgen receptor pathway in Sertoli cells is vital for complete spermatogenesis  abs#102

10:45am  
Kirsten McTavish
Evidence that premature infertility in transgenic FSH female mice is due to age-related changes in embryo survival: ageing oocyte or uterus?  abs#103

11:00am  
Christina Jang
Reduced 11β Hydroxysteroid Dehydrogenase-1 in Skeletal Muscle in type 2 Diabetes: Induction by Dexamethasone  abs#104

11:15am  
Rachael O'Dowd
Postnatal growth is improved by cross-fostering a pup born small onto a mother with normal lactation by altering alveolar area, milk production and milk protein gene expression  abs#105

11:30am  
Sean Yang
Regulation of voltage-gated Ca$^{2+}$ currents of rat somatotropes through subtypes of somatostatin receptors  abs#106

SRB Orals - Pregnancy and Fetus
10:15 AM - 12:00 PM  
M5
Co-Chair: Larry Chamley and Guiying Nie

10:15am  
Viv Perry
The effect of maternal protein during pregnancy on birth weight in the bovine  abs#215

10:25am  
Brandon Menzies
Distribution of ghrelin secreting cells in the stomach of the developing marsupial, Macropus eugenii  abs#217

10:35am  
Lenka Vodstrcil
Restricting uterine blood flow in late pregnant rats results in an increase in uterine relaxin receptor (Lgr7) expression  abs#218

10:45am  
Sarah Robertson
Systemic maternal awareness of conceptus antigens in pregnancy  abs#219

10:55am  
Katrina Hadfield
Investigation of Maternal Immune Function Throughout Pregnancy  abs#220

11:05am  
Claire Roberts
Maternal insulin-like growth factor-I and -II act via different pathways to increase fetal growth near term  abs#221

11:15am  
Amanda Sferruzzi-Perri
Maternal IGF treatment in early to mid pregnancy has sustained effects on placental transport & nutrient partitioning near term  abs#222

11:25am  
Kathryn Hale
HtrA3, a serine protease, in human pregnancy serum.  abs#223
SRB Orals - Growth Factors and Signalling
10:15 AM - 12:00 PM
Co-Chair: Ravinder Anand-Ivell and Wendy Ingman

10:15am Ravinder Anand-Ivell
Characterizing the relaxin receptor (RXFP1) and the relaxin signaling pathway in human uterine cells.  abs#224

10:25am Stephen Anderson
Increased expression of suppressors of cytokine signalling (SOCS) in the rat ovary during pregnancy  abs#225

10:35am Mai Sarraj
Expression of Follistatin like -3 in Developing Mouse Gonads  abs#226

10:45am Laura Parry
The lethal phenotype in relaxin-deficient (Rlx-/-) mice is due to abnormal nipple growth, and not impaired mammary gland structure or function.  abs#227

10:55am Wendy Ingman
Null mutation in transforming growth factor beta1 impairs mammary gland development and impedes lactation  abs#228

11:05am Jinwei Chung
Insulin family receptors in the developing marsupial  abs#229

11:15am Anette Szczepny
Disruption of Hedgehog signalling in the adult mouse testis  abs#230

11:25am Mark Hedger
Regulation of spermatogonial proliferation by interleukin-1 and activin A in vitro: a re-examination using an antagonist approach  abs#231

SRB Founders Lecture
12:00 PM - 1:00 PM
Chair: Lois Salamonsen

Marilyn Renfree
Life in the pouch: Womb with a view  abs#005

Session sponsored by RFD

ESA Clinical - Meet the Expert 1: Vitamin D Across the Ages
12:00 PM - 1:00 PM
Chair: Roderick Clifton-Bligh

Terry Diamond
Vitamin D across the ages  abs#006

Session sponsored by GSK

Lunch
1:00 PM - 2:00 PM
Exhibition Hall

Session sponsored by Novo Nordisk

ANZPRA AGM
1:15 PM - 2:00 PM

ESA Monday Poster Session - Pituitary, Reproduction, Pregnancy, Clinical I
2:00 PM - 3:00 PM
Central BC

For poster listing, see end of Monday
SRB / ANZPRA Symposium - Growth Factor Regulation of Implantation and Placental Development

2:00 PM - 3:30 PM  M5
Co-Chair: Claire Roberts and Padma Murthi  
Session sponsored by Eli Lilly

2:00pm  Claire Roberts  
The pivotal role of IGF-II in placental invasion, growth and function  abs#007

2:30pm  Euan Wallace  
Macrophage inhibitory cytokine-1: roles in trophoblast function and decidual preparation  abs#008

3:00pm  Evdokia Dimitriadis  
Interleukin 11 and leukemia inhibitory factor: mechanisms and interactions in implantation and placentation  abs#009

SRB Orals - Endocrine Regulation of Reproductive Function

2:00 PM - 3:30 PM  M6
Co-Chair: Christopher Grupen and Chris Scott

2:00pm  Penelope Hawken  
Ram introduction stimulates pulsatile LH secretion in cyclic ewes  abs#232

2:10pm  Eva Szarek  
Development of anterior pituitary cells and colocalisation of TSHβ and FSHβ with LHβ-immunoreactivity in the late gestational sheep fetus after disconnection of the hypothalamus and pituitary  abs#233

2:20pm  Chris Scott  
Differentiating the sites of action of testicular steroids in the regulation of GnRH secretion and mating behaviour in rams: A model  abs#234

2:30pm  Susan Jones  
Modulation of adrenal responsiveness in gestating females of Egernia whitii, a viviparous skink  abs#236

2:40pm  Emily Hynes  
The contraceptive mechanism of levonorgestrel in a marsupial species  abs#237

2:50pm  Christopher Grupen  
An effective superovulation protocol for the marmoset monkey (Callithrix jacchus)  abs#238

3:00pm  David Armstrong  
Prostaglandin E₂ up-regulates luteinizing hormone receptor (LHR) expression and enhances steroidogenic responses of follicle cells  abs#239

ESAs Basic Symposium - Nuclear Receptor Regulation and Endocrine Disease

3:00 PM - 5:00 PM  M7
Co-Chair: Anne Nelson and Charles Allan

3:00pm  Peter Fuller  
Determinants of tissue and ligand selectivity in the mineralocorticoid receptor  abs#010

3:30pm  Colin Clyne  
Roles of orphan receptor LRH-1 in reproduction and cancer  abs#011

4:00pm  Edith Gardiner  
Vitamin D-Wnt pathway interactions in skeletal and non-skeletal cells  abs#012

4:30pm  Gary Leong  
Nuclear receptors in metabolism: the Ski phenotypes and the NORphans  abs#013

ESAs Clinical Symposium - Pituitary disease: A Wide Spectrum of Disorders

3:00 PM - 5:00 PM  Arena 1B
Co-Chairs: Shaun McGrath and Roderick Clifton-Bligh  
Session sponsored by Ipsen

3:00pm  Warrick Inder  
Aspects in the diagnosis and management of prolactinoma  abs#014

3:30pm  Ken Ho  
Acromegaly: new frontiers in management  abs#015

4:00pm  Steven Santorenus  
Pituitary surgery with a pituitary tumour focus  abs#016
4:30pm  Mark McLean
Insights into monitoring therapy, and challenges in management of hypopituitary patients  abs#017

ESA Orals - Diabetes, Obesity
3:00 PM - 5:00 PM
Co-Chairs: David Kennaway and Brendan Waddell

3:00pm  Caitlin Wyrwoll
A postnatal diet rich in omega-3 fatty acids attenuates glucocorticoid-programmed hyperinsulinemia but does not alter aberrant programmed gene expression in skeletal muscle.  abs#107

3:15pm  David Kennaway
A lack of peripheral tissue rhythmicity alters metabolic homeostasis in mice  abs#108

3:30pm  Chen Chen
Free fat acids (FFAs) stimulate Ca^{2+} release from IP_{3}-sensitive Ca^{2+} storage sites and reduce voltage-gated Ca^{2+} currents in primary cultured rat pancreatic \beta-cells  abs#109

3:45pm  Helena Parkington
Regional differences in endothelial dysfunction in diabetes mellitus  abs#110

4:00pm  Nichola Thompson
Preference between exercise and eating is influenced by prenatal nutrition and obesity development is prevented by providing an opportunity to exercise.  abs#111

4:15pm  Jenny Chow
The effect of estrogen on triglyceride homeostasis  abs#112

4:30pm  Michele Gresham
Circulating Porcine Ghrelin Concentrations are Responsive to Energy Metabolites and not Insulin  abs#113

ESA / SRB Orals - Joint Female Reproduction
4:00 PM - 6:00 PM
Co-Chairs: Simon Chu and Laura Parry

4:00pm  Ashwini Chand
Inhibin \alpha subunit with an A257T mutation is associated with premature ovarian failure: Is this inhibin form bioactive?  abs#240

4:15pm  Tu'uhwahara Kaitu'u-Lino
Neutrophil depletion retards endometrial repair  abs#241

4:30pm  Kristy Brown
Hydroxysteroid Sulfoconjugation as a Putative Determinant of Follicular Luteinization  abs#242

4:45pm  Christine White
Novel leukemia inhibitory factor antagonist blocks blastocyst implantation in the mouse  abs#243

5:00pm  Ashwini Chand
Laser Capture Microdissection and Array Analysis of Endometrium Identify CCL16 and CCL21 as Epithelial - Derived Inflammatory Mediators Associated with Endometriosis  abs#244

5:15pm  Yin Lau Lee
Hormonal Regulation and Convertase activities of complement 3 in human oviductal epithelial cells  abs#245

5:30pm  Simon Chu
The Role of Imatinib in the regulation of Granulosa Cell Tumour cell growth  abs#246a

5:45pm  Grant Montgomery
Novel variants in human GDF9 in mothers of dizygotic twins  abs#246b
SRB Orals - Spermatogenesis and Sperm
4:00 PM - 6:00 PM
Co-Chairs: Julia Young and Steve Johnston

4:00pm  Jennifer Ly
Importin α2-recognised nuclear import in the control of spermatogenesis  abs#247

4:10pm  Kim Lieu
The importin-α2-dependent nuclear import mechanism of PSMC3IP and its possible role during spermatogenesis  abs#248

4:20pm  Alison Graham
Coexpression and potential interaction of the nuclear transporter importin β3 and nuclear pore complex component nucleoporin Nup153 in mouse testis.  abs#249

4:30pm  Vinali Dias
Differential expression of activin receptors in normal, hormone-treated, and neoplastic human testis.  abs#250

4:40pm  Minjie Lin
Ontogeny of cAMP-dependent tyrosine phosphorylation-signaling pathways during spermatogenesis and epididymal maturation in the mouse  abs#251

4:50pm  Jeanette Olejnik
PGP 9.5 as a marker for germ cell development in pre-pubertal and irradiated sheep testes.  abs#252

5:00pm  Brian Setchell
Long-term effects on the testis of a short period of unilateral cryptorchidism in rats  abs#253

5:10pm  YengPeng Zee
Assessment of Koala sperm mitochondrial function with JC-1  abs#254

5:20pm  Steve Johnston
One-sided ejaculation of sperm bundles in the echidna  abs#255

5:30pm  Phillip Matson
The objective assessment of porcine epididymal sperm using the sperm quality analyzer IIB.  abs#256

SRB Post Doc Meeting
6:00 PM - 6:30 PM  M5

ESA AGM
6:00 PM - 6:30 PM  M7

SRB Student Meeting
6:00 PM - 6:30 PM  M6

ESA / SRB Student Function
7:00 PM - 12:00 PM  BOJANGLES, Broadbeach

FUTURE MEETINGS

2006
SRB Symposium at the 3rd Australian Health and Medical Research Congress “Inflammation and immunity: the oft-neglected challenge for reproductive health”
28th November 2006
Melbourne Convention Centre, VIC
www.ahmrcongress.org.au

2007
Combined ESA/SRB Annual Scientific Meeting
2nd – 5th September 2007
Christchurch Convention Centre, NZ
www.esa-srb.org.au
SRB MLA Plenary Lecture
8:30 AM - 9:30 AM  
Chair: Graeme Martin  
Session sponsored by Meat Livestock Australia

**William Thatcher**  
Nutraceutical and pharmaceutical effects on uterine and hormonal responses associated with early pregnancy in lactating dairy cattle  
*abs#018*

**ESA Clinical - Meet the Expert 2: Iodine Sufficiency Across Australia: Do we currently make the grade?**

8:30 AM - 9:30 AM  
Chair: John Walsh

**Creswell Eastman**  
Iodine sufficiency across Australia: do we currently make the grade?  
*abs#019*

**ESA Orals - Pregnancy, Parturition**

8:30 AM - 9:30 AM  
Co-Chairs: Vicki Clifton and Julie Owens

8:30am **Elisa Tyson**  
A novel mode of action for oxytocin and cAMP in regulating myometrial contractility  
*abs#123*

8:45am **Kerryn Westcott**  
Growth restricted fetal and newborn rats have altered brain neurosteroids  
*abs#124*

9:00am **Mary Wlodek**  
Puberty onset is delayed following placental and lactational restriction  
*abs#125*

9:15am **Julie Owens**  
Restriction of placental growth and size at birth increases pancreatic expression of the β-cell survival factor IGF-II.  
*abs#126*

**Morning Tea**

9:30 AM - 10:00 AM  
Exhibition Hall

**ESA / SRB Orals - Joint Male Reproduction Session**

10:00 AM - 12:00 PM  
Co-Chairs: Kate Loveland and Mark Hedge

10:00am **Ulla Simanainen**  
Prostate atrophy and abnormal epithelial cell proliferation due to targeted disruption of the prostate epithelial androgen receptor in PEARKO mice  
*abs#258a*

10:15am **Geoffrey Shaw**  
Androstanediol and development of the Wolffian ducts in tammars  
*abs#258b*

10:30am **Yao Wang**  
Anti-Activin consequences of glucocorticoid action within the male reproductive axis  
*abs#259*

10:45am **Gurpreet Kaur**  
Calmodulin-dependent nuclear import of the testis-determining factor SRY  
*abs#260*

11:00am **Jayne Sierens**  
Liver Receptor Homologue-1 (LRH-1) regulated genes within the testis.  
*abs#261*

11:15am **Elspeth Gold**  
Activin βC-subunit is a regulator of testis and liver function: implications for activin biology  
*abs#262*

11:30am **Yogeshwar Makanji**  
Glycosylated forms of human inhibin A and B show marked differences in *in vitro* bioactivity  
*abs#263*

11:45am **Gerard Tarulli**  
In vivo regulation of tight junction proteins by gonadotrophins in the adult Djungarian hamster testis  
*abs#264*
ESA Orals - Transcription, Signalling
10:00 AM - 12:00 PM  Central A
Co-Chairs: Esme Hatchell and Gary Leong
10:00am  **Stephen Myers**  
Crosstalk between the chicken ovalbumin upstream promoter transcription factors (COUP-TFs) and LXR in skeletal muscle cells regulates lipid homeostasis  abs#143
10:15am  **Carolyn Mitchell**  
Chromatin structure and NFκB binding in the prostaglandin endoperoxide H synthase (PGHS-2) promoter in term fetal membranes  abs#144
10:30am  **Brendan Waddell**  
Interactive effects of fetal programming and postnatal dietary omega-3 fatty acids on methylation status of renal glucocorticoid receptor and 11β-hydroxysteroid dehydrogenase 2.  abs#145
10:45am  **Esme Hatchell**  
Complex interactions between SLIRP, A SRA-binding nuclear receptor corepressor, and other nuclear receptor coregulators  abs#146

**ESA Orals and Clinical Session: ESA Mayne Pharma Bryan Hudson Clinical Award**  
- *the first two session presenters are Award Finalists*
10:00 AM - 12:00 PM  M8
Co-Chairs: Carolyn Allen and Mark McLean
10:00am  **Peter Liu**  
The rate, extent and modifiers of spermatogenic recovery after male hormonal contraception: an integrated analysis  abs#151
10:15am  **Jui Ho**  
Relative maternal hypocortisolism in high risk human pregnancy  abs#152
10:30am  **Roger Smith**  
Rate of change of corticotrophin releasing hormone and hCG nadir provide accurate identification of women at risk of preterm birth  abs#153
10:45am  **Florence Law**  
Proton pump inhibitors cause hypomagnesaemic hypoparathyroidism  abs#154
11:00am  **John Walsh**  
Small changes in thyroxine dosage do not produce measurable changes in hypothyroid symptoms, well-being or quality of life: results of a double blind, randomized clinical trial.  abs#155
11:15am  **Peter Ebeling**  
Pamidronate or zoledronic acid reduce bone loss after allogeneic stem cell transplantation  abs#156
11:30am  **Jun Yang**  
Multifocal papillary thyroid carcinoma arising in Hashimoto's thyroiditis  abs#157
11:45am  **Paul Lee**  
Hyperserotoninaemia in Gilbert's syndrome mimicking carcinoid syndrome - A novel mechanism?  abs#158
**ESA Orals - HPA, Pituitary**
10:00 AM - 12:00 PM  
Co-Chairs: Catherine Coulter and Duncan Topliss

10:00am  **Timothy Cole**  
Direct effects of aldosterone *in vivo* on endothelin-1 gene expression in the kidney and colon  
*abs#127*

10:15am  **Catherine Coulter**  
Maternal dexamethasone treatment in early gestation suppresses steroidogenic capacity and growth of the fetel adrenal  
*abs#128*

10:30am  **Matthew Doogue**  
Salivary cortisol to monitor hydrocortisone treatment in patients in patients with hypoadrenalism  
*abs#129*

10:45am  **Peter Mark**  
Elevated P-glycoprotein expression limits glucocorticoid receptor response to cortisol and impedes dexamethasone transport across monolayers in placental BeWo cells  
*abs#130*

11:00am  **Craig Harrison**  
Activin-A binds follistatin and type II receptors through overlapping binding sites: Generation of mutants with isolated binding activities  
*abs#131*

11:15am  **John Fitter**  
Different environmental stresses elicit differential CRH responses in limnodynastes peronii  
*abs#132*

11:30am  **Shoshana Sztal-Mazer**  
Functional FSH-secreting adenoma in MEN1  
*abs#133*

11:45am  **Rachel Hill**  
Lack of estrogen leads to a significant reduction in area and cell number of a region corresponding to the Sexually Dimorphic Nucleus (SDN) of the medial preoptic area in male and female mice of the Sv129J strain  
*abs#134*

**SRB Symposium - Stem Cells in Reproductive Tissues**
10:00 AM - 12:00 PM  
Chair: Caroline Gargett and Renea Taylor  
Session sponsored by Australian Stem Cell Centre

10:00am  **Melissa Little**  
Searching for stem cells in the kidney  
*abs#020*

10:30am  **Jane Visvader**  
Identification of mammary stem cells and their role in breast cancer.  
*abs#021*

11:00am  **Jock Findlay**  
Germline stem cells in the ovary  
*abs#022*

11:30am  **Caroline Gargett**  
Adult stem/progenitor cells in the endometrium  
*abs#023*

**ESA Harrison Lecturer**
12:00 PM - 1:00 PM  
Chair: Jeffery Zajac

**William Crowley**  
New genes that control reproduction in the human and their genotype-phenotypes: Evidence from human disease models  
*abs#024*

**SRB lunch including Student Lunch - with the Founder's Lecturer**
12:00 PM - 1:00 PM  
Collect lunch first from the Exhibition Hall

**ESA Lunch**
1:00 PM - 2:00 PM  
Session sponsored by Eli Lilly
SRB Orals - Oocytes and Ovarian Function
1:00 PM - 3:00 PM
Co-Chairs: Ray Rodgers and Ann Drummond
1:00pm  Raymond Rodgers
Gene expression profiling by microarray analyses of bovine granulosa cells from small and large healthy antral follicles  abs#265

1:10pm  Helen Irving-Rodgers
Correlative gene expression and follicular dominance in the bovine  abs#266

1:20pm  Ann Drummond
Mechanism by which FGF9 stimulates ovarian progesterone production  abs#267

1:30pm  Chris Edgecumbe
The localisation of cyclin B1 and fat facets in mouse (FAM) in murine oocytes undergoing maturation in vitro  abs#268

1:40pm  Cheryl Schelbach
Improved development of murine embryos derived from COCs matured with the O-linked glycosylation inhibitor, BADGP.  abs#269

1:50pm  Kelly Banwell
Fetal and placental outcomes are programmed by oxygen concentration during maturation of murine oocytes.  abs#270

2:00pm  Michael Boden
Changes in ovarian gene expression and mammary development in the BMAL1 knockout mouse  abs#271

2:10pm  Jean Fleming
Kallikrein 4 expression in mouse ovaries with serous inclusion cysts.  abs#272

2:20pm  Marissa Bowden
Expression of Serine Protease Htra3 During Ovarian Development and Folliculogenisis in the Rat  abs#273

2:30pm  Carolina Viñoles
Use of a single-follicle-wave cycle to study acute effects of changes in nutrition on ovulation rate in ewes  abs#274

SRB Orals - Uterus and implantation
1:00 PM - 3:00 PM
Co-Chairs: Eva Dimitriadis and John Bromfield
1:00pm  Alejandro Tapia
Identification of genes in human endometrial and stromal cells that alter during the acquisition of receptivity  abs#275

1:10pm  Chelsea Stoikos
Activin inhibitors retard human endometrial stromal cell decidualisation  abs#276

1:20pm  Laura Lindsay
Fluid transport in the rat uterus during early pregnancy  abs#277

1:30pm  Joanna Biazik
Evolution of viviparity in reptiles: role of tight junctions of the uterine epithelium  abs#278

1:40pm  John Bromfield
Seminal plasma regulates MMP-2, MMP-3 and VEGF-C mRNA expression in the peri-implantation mouse uterus  abs#279

1:50pm  Shalini Panwar
Leptin is critical for successful implantation in mice  abs#280

2:00pm  Claudia Freyer
Pro-protein convertases: novel regulators of endometrial physiology and implantation  abs#281

2:10pm  Lynnette Kilpatrick
Identification of pc6 substrates involved in human stromal cell decidualisation using proteomics  abs#282

2:20pm  Anita Ledgard
Effect of polysaturated fatty acids on PGF 2α and PGE 2 synthysis in bovine endometrium and trophoblast tissues.  abs#283

2:30pm  Claire Walker
The distribution of opioid receptors in the human placenta and decidua from early pregnancy  abs#284
Combined Neuroendocrinology Australasia /ESA Symposium:  
Kisspeptins in Neuroendocrinology

3:00 PM - 5:00 PM  
Chair: Brian Oldfield  
Session sponsored by DSL Australia / Beckman Coulter Australia

3:00pm Allan Herbison  
Kisspeptin activation of GnRH neurons to initiate puberty and ovulation.  
abs#025

3:30pm William Crowley  
The biology of kisspeptins and GPR54  
abs#026

4:00pm Manuel Tena-Sempere  
Kisspeptin and GPR54: molecular conduits for puberty onset and central integration of energy balance and reproduction  
abs#027

4:30pm Jeremy Smith  
The role of kisspeptin in mediating sex steroid feedback control of GnRH  
abs#028

ESA Orals - Androgens, Cancer

3:00 PM - 5:00 PM  
Co-Chairs: Helen MacLean and Judith Clements

3:00pm Lisa Butler  
Suberoylanilide hydroxamic acid (SAHA), a histone deacetylase inhibitor, represses androgen receptor expression and acts synergistically with an androgen receptor antagonist to inhibit prostate cancer cell proliferation  
abs#159

3:15pm Tina Bianco-Miotto  
Expression of the androgen receptor and its association with disease outcome in breast cancer  
abs#160

3:30pm Sharyn Kelleher  
Older men with organic androgen deficiency (ad) maintain similar trough and peak blood testosterone levels and quality of life as younger AD men without change in testosterone implant dose.  
abs#161

3:45pm Angela Chew  
Mechanisms underlying inhibition of androgen-insensitive prostate cancer cell proliferation by peroxisome proliferator-activated receptor gamma.  
abs#162

4:00pm Maggie Centenera  
Development of dominant negative androgen receptors as a novel therapeutic strategy for prostate cancer  
abs#163

4:15pm Helen MacLean  
Global and muscle-specific androgen receptor knockout mice demonstrate direct anabolic actions of androgens in skeletal muscle  
abs#164

4:30pm Mathis Grossmann  
Low testosterone levels are not common in men with type 2 diabetes mellitus and, in contrast to low SHBG levels, not associated with poor glycaemic control.  
abs#165

AACB / ESA clinical symposium - Laboratory measures in clinical endocrinology practice

3:00 PM - 5:00 PM  
Co-Chairs: Gregory Ward and Shaun McGrath  
Session sponsored by Servier

3:00pm Paul Glendenning  
Laboratory measures in clinical endocrine practice: Bone markers  
abs#029

3:30pm David Handelsman  
Testosterone measures - integration of clinical and analytical issues  
abs#030

4:00pm Paul Williams  
Macroprolactin  
abs#031

4:30pm Jim Stockijt  
Thyroid antibodies in clinical endocrinology  
abs#032
Afternoon Tea (SRB)
3:00 PM - 3:30 PM
Exhibition Hall

SRB Young Investigators
3:30 PM - 5:00 PM
Central A
Chair: Lois Salamonsen
Session sponsored by Serono

3:30pm  Rachel Chan
Estrogen stimulates mouse endometrial stem-like cell proliferation  abs#285

3:40pm  Natalie Hannan
A novel role for fractalkine in regulating human trophoblast extracellular matrix and adhesion molecules.  abs#286

3:50pm  Premila Paiva
Interleukin-11 promotes migration but not proliferation of human trophoblast cells  abs#287

4:00pm  Saleela Ruwanpura
Accelerated apoptosis is the cause of germ cell loss in gonadotrophin-suppressed men  abs#288

4:10pm  Kirsty Walters
Androgen receptor is not essential for female reproduction but plays important roles in optimising follicle development and ovulation  abs#289

4:20pm  Camryn Allen
Successful artificial insemination (AI) in the koala using neat and extended semen collected by electroejaculation (EE)  abs#290

ESA Lecture
5:00 PM - 6:00 PM
Arena 1B
Chair: Leon Bach

Peter Leedman
Nuclear receptor coregulators – getting to the heart of hormone action  abs#033

ESA / SRB Conference Dinner - Gold Coast Convention Centre
7:30 PM - 12:00 PM
Foyer E upstairs
Wednesday, 23 August 2006

ESA & ADS Plenary
8:30 AM - 9:30 AM  
Chair: Terri Allen and Stephen Twigg

Eiichi Araki
Intracellular stress, mitochondrial dysfunction and diabetes complications  abs#034

SRB Orals - Infertility and Pregnancy Pathologies
8:30 AM - 9:30 AM  
Co-Chairs: Jane Girling and Louise Hull

8:30am  Naomi Morison
Mifepristone enhances endometrial repair in a mouse model for break-through bleeding associated with implanon use.  abs#291

8:40am  Kylie Van der Hoek
Osteoblast specific factor -2 and osteopontin are present in endometriotic tissues.  abs#292

8:50am  Kathryn Visser
Endometrial interleukin 11 is dysregulated in infertility during the implantation window.  abs#293

9:00am  Rachael Nowak
Interleukin-1β -511 polymorphism is associated with preeclampsia  abs#294

9:10am  Katy Freed
The gene encoding the constant region of the heavy chain of immunoglobulin G is differentially expressed in human decidua in association with preeclampsia  abs#295

9:20am  Larry Chamley
The role of the novel sperm protein SPRASA in infertility  abs#296

SRB Orals - Germ Cells and Stem Cells
8:30 AM - 9:30 AM  
Co-Chairs: Peter Koopman and Rachael Chan

8:30am  Julia Young
The influence of regulated nuclear transport on primordial germ cell lineage specification  abs#297

8:40am  Danielle Hickford
Primordial germ cell specification: an alternative mammalian model.  abs#298

8:50am  Zhen Zhang
Establishment of molecular markers for bovine testis transplantation  abs#299

9:00am  Muren Herrid
Magnetic activated cell sorting for purification of bovine type A spermatogonia  abs#300

9:10am  Sirisha Mendis
Activin effects germ cell number at specific developmental ages in the fetal mouse testis  abs#301

9:20am  Sridurga Mithra-Prabhu
Regulation of c-Kit receptor in germ cells of the rodent testis by members of TGF-beta superfamily  abs#302

Morning Tea
9:30 AM - 10:00 AM  
Exhibition Hall

ESA / ADS Symposium - Routes of Hormonal/Insulin Delivery
10:00 AM - 12:00 PM  
Chair: Paul Williams and Richard McIsaac

Session sponsored by Schering

10:00am  Eiichi Araki
Novel approaches for the treatment of Diabetes -wearable artificial endocrine pancreas (AEP) and mild electric and thermo generator (MET)-  abs#035

10:30am  Dennis Yue
Inhaled insulin in diabetes treatment  abs#036

11:00am  Ann Simpson
Insulin delivery by genetic engineering  abs#037
11:30am  Ann Conway  
Testosterone delivery - routes of administration  abs#038

ESA / SRB Symposium - Genetic Causes of Reproductive Failure
10:00 AM - 12:00 PM  
Co-Chairs: Guiying Nie and Robert Norman
10:00am  William Crowley  
Hypothalamic causes of reproductive behaviour  abs#039
10:30am  Kate Steinbeck  
Turner Syndrome  abs#040
11:00am  Moira O’Bryan  
Genetic causes of reproductive failure in the male  abs#041
11:30am  Guiying Nie  
Endometrial proprotein convertase 6: a critical regulator for embryo implantation  abs#042

ESA Orals - Growth Hormone, IGFs, IGFBPs
10:00 AM - 12:00 PM  
Chair: Vince Russo and Kin Keung
10:00am  Rebecca Pelekanos  
The growth hormone receptor is constitutively dimerized and activated by rotation of the cytoplasmic domains  abs#166
10:15am  Linda Kerr  
Removal of Box1 from the GH Receptor abolishes Jak/STAT signalling but not MAPK  abs#167
10:30am  Kin-Chuen Leung  
Contrasting regulatory effects of selective oestrogen receptor modulators on GH signalling in breast and kidney tissues  abs#168
10:45am  Kathryn Gatford  
Reduced IGFBP-3 abundance contributes to catch-up growth in the intrauterine growth-restricted lamb.  abs#169
11:00am  Vincenzo Russo  
Insulin-like growth factor binding protein-2 is an essential regulator of neuroblastoma cell motility  abs#170
11:15am  Kin-Chuen Leung  
GH regulation of metabolic genes in muscle: A microarray study in hypopituitary men  abs#171
11:30am  Anne Nelson  
The influence of demographic factors on the ratio of 20-kDa and 22-kDa GH isoforms and the utility of the ratio for detection of GH doping in sport.  abs#172
11:45am  Vita Birzniece  
Modulatory effect of raloxifene and oestrogen on the metabolic action of GH in hypopituitary women  abs#173

SRB Orals - Embryo Development
10:00 AM - 12:00 AM  
Co-Chairs: Marie Pantaleon and Michelle Lane
10:00am  Christine Yeo  
Exogenous growth differentiation factor 9 during in vitro maturation of oocytes improves subsequent embryonic development and fetal outcome  abs#303
10:10am  W.N. Chow  
Fertility study of complement-3 in mice  abs#304
10:20am  Chris O’Neill  
Variable expressivity of the tumour suppressor protein P53 in human embryos  abs#305
10:30am  Hugh Morgan  
Demonstration of an association between the extent of culture stress and the expression of P53 in mouse embryos.  abs#306
10:40am  Vikram Tallapragada  
P53 expression in mouse and human sperm  abs#307
10:50am  Don Rieger  
Comparison of a single medium with sequential media for the culture of sibling human embryos to the blastocyst stage  abs#308

11:00am  Celine Lawler  
Significance of early developmental markers in human cryopreserved embryos  abs#309

11:10am  Erica Little  
Characterization of E74 like factor 3 in the murine blastocyst  abs#310

11:20am  Melanie Gibson  
The investigation of mRNA expression of nuclear importins during bovine preimplantation embryogenesis.  abs#311

Lunch  
12:00 PM - 1:00 PM  
Exhibition Hall

SRB / DE Symposium - Epigenetic Mechanisms in Programming Pre-implantation Embryos  
1:00 PM - 2:30 PM  
M7 & M8  
Chairs Chris O'Neil and Sarah Robertson  
Session sponsored by ARC/NHMRC Network in Genes and Environment in Development

1:00pm  Lorraine Young  
Environmental influences on DNA methylation in embryonic cells: investigating mechanisms and phenotypic consequences.  abs#043

1:30pm  Josie McConnell  
A mitochondrial component to developmental programming  abs#044

2:00pm  Hugh Morgan  
Methylcytosine deamination by DNA deaminases and expression in reprogramming tissues.  abs#045

Downunder Embryo Symposium continues  
1:00 PM - 5:00 PM  
M7
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001

THE ROLE OF NON-CODING RNA IN HUMAN DEVELOPMENT
J. S. Mattick
Institute for Molecular Bioscience, University of Queensland, St. Lucia, QLD, Australia

It appears that we have fundamentally misunderstood the nature of genetic programming in humans and other multicellular organisms for the past 50 years because of the presumption, largely correct in prokaryotes but not in complex eukaryotes, that most genetic information is transacted by proteins, which form the main analog components of all cells. Humans have the same number of protein coding genes (19,500) as the nematode worm (~19,300), which has only 1,000 cells. Although only 1.2% of the human genome encodes proteins, the vast majority is actually transcribed in a developmentally regulated fashion, much of it on both strands. These transcripts include tens if not hundreds of thousands of small RNAs, including miRNAs, snoRNAs, piRNAs and other yet-to-be-discovered classes of regulatory RNAs, many of which are encoded in introns, and longer noncoding RNAs that exhibit dynamic expression patterns during germ cell and ES cell differentiation, gonadal development, muscle development, brain development, and macrophage and T-cell activation, to name a few. Many are dysregulated in disease, including neurological diseases and cancer. It is also now evident that most, if not all, complex genetic phenomena in the higher organisms are directed by RNA signaling pathways. Taken together, the data suggest that most of the human genome and those of other complex organisms, including transposon-derived sequences, is not junk nor evolving neutrally, but rather encodes a hitherto hidden layer of regulatory RNAs (many of which are species- or lineage-specific) that set the settings and direct the trajectories of differentiation and development via the control of chromatin architecture and epigenetic memory, promoter selection, splicing, RNA modification and editing, and mRNA stability and translation.


002

WHY MEN MAKE SPERM AND WOMEN MAKE OOCYTES: DISCOVERY OF THE MOLECULAR SIGNALS CONTROLLING GERM CELL FATE DURING EMBRYONIC DEVELOPMENT
J. Bowles1, D. Knight1, C. Smith1, M. J. Wilson1, D. Wilhelm1, J. Richman1,2, S. Mamiya3, K. Yashiro3, K. Chawengsaksophak4, J. Rossant1, H. Hamada1, P. Koopman1

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In mouse embryos, germ cells in developing ovaries enter meiosis and begin oogenesis around 13.5 dpc, whereas those in male gonads cease dividing until after birth, signaling a spermatogenic fate. It is widely believed that germ cells are intrinsically programmed to enter meiosis at a predetermined time, unless prevented from doing so by factors secreted by the male gonad. Instead, we now find that retinoic acid (RA) signaling controls the nexus between spermatogenesis and oogenesis.

We conducted an expression screen designed to identify genes expressed in a male- or female-specific manner during mouse gonadogenesis, and identified two genes encoding enzymes involved in RA metabolism. We detected abundant RA production in the adjacent mesonephros of both sexes; the RA diffuses into the gonads in both sexes, persisting at high levels in the ovary, but is cleared from developing male gonads by the degradative enzyme Cyp26B1. By treatment of gonadal explants, we showed that either retinoic acid or an inhibitor of CYP26B1 induces XY germ cells to enter meiosis. Conversely, a retinoic acid receptor antagonist blocks entry of XX germ cells into meiosis. Meiotic markers were also induced in testes of Cyp26b1 knockout mice. Together, our data suggest that retinoic acid, produced by the mesonephros, induces germ cells in the female gonad to enter meiosis but is prevented from doing so in the male gonad because of the actions of CYP26B1. Our data identify RA as a molecular trigger of meiosis in fetal gonads, a discovery that may be applicable to modulating human or animal fertility in vivo and production of functional gametes from germline stem cells in vitro.
A NOVEL APPROACH TO STUDY HUMAN PROSTATE DEVELOPMENT AND ITS DISEASES

G. Risbridger, R. Taylor, P. Cowin

Centre for Urological Research (CURE), Monash Institute of Medical Research, Monash University, VIC, Australia

Men are not mice and rodent models have limited utility and relevance when studying human diseases. This is particularly true in the study of prostate disease, both benign and malignant, since these do not occur in mice. Yet mice are commonly used for this purpose and rodent models have provided controversial evidence for the early origins of adult prostate disease that is almost impossible to verify in humans. How do we develop model systems of human disease?

Our approach was to use the classical biological technique of tissue recombination together with stem cell technology to generate non-diseased human prostate tissue. We used rodent mesenchyme to establish reciprocal stromal-epithelial cell interactions with human ESC and directed their differentiation to fetal and mature human prostate glands, expressing PSA (prostate specific antigen), within 12 weeks; a process that takes 15 years or more in men. Glands derived from hESC of different genetic sex, first express fetal markers of prostate differentiation (eg Nkx3.1), followed by markers of its maturation (eg AR, p63 and PSA) and all the tissues are hormonally responsive. This model provides new opportunities to study prostate disease. Firstly it provides normal tissue that is only available from young men aged 20-30yo; from this normal tissue, the process of disease initiation and progression can be studied, especially cancer. The mechanism of disease induction can be explored and verified by up or down regulating specific gene expression in the mesenchyme or stroma, eg using genetically modified mouse tissue or siRNA. Further, since fetal tissues are generated, we are able to study the early origins of adult disease, specifically the transgenerational effects of endocrine disruptors.

The conservation of stromal-epithelial signalling mechanisms between rodent and human species suggests this approach could extend to integumental, gastrointestinal and genital tissues, enabling the development of more relevant models of human diseases.

LIFE IN THE POUCH: WOMB WITH A VIEW

M. B. Renfree

Zoology, The University of Melbourne, Melbourne, VIC, Australia

Marsupials give birth to altricial young after a relatively short gestation period, but have a long and sophisticated lactation while the young develop, usually within a pouch. Their viviparous mode of reproduction thus minimises placentaion in favour of lactation, effectively trading the umbilical cord for the teat. The special adaptations that marsupials have developed provide us with unique insights into the evolution of mammalian reproduction. Marsupials hold many mammalian reproductive “records”, for example they have the shortest known gestation but the longest embryonic diapause; the smallest neonate but the longest sperm. They have contributed to our knowledge of many mammalian reproductive events including embryonic diapause and development, birth behaviour, sex determination, sexual differentiation, lactation and seasonal breeding. Since marsupials have been genetically isolated from eutherian mammals for over 125 million years, sequencing of the genome of two marsupial species has made comparative genomics an exciting and important new area of investigation. This review will show how the study of marsupials has widened our understanding of mammalian reproduction and development, highlighting some of the mechanisms that are so fundamental that they are shared by all today's marsupial and eutherian mammals.

THE PIVOTAL ROLE OF IGF-II IN PLACENTAL INVASION, GROWTH AND FUNCTION

C. T. Roberts

Research Centre for Reproductive Health, Obstetrics and Gynaecology, University of Adelaide, Adelaide, SA, Australia

The placenta has a myriad of functions including exchange of oxygen, nutrients and wastes between the maternal and fetal circulations. In early pregnancy, placental trophoblast cells invade and colonise the decidua and its vasculature to sequester a blood supply for the growing placenta. Impairments in this process have been implicated in pregnancy complications including IUGR, preeclampsia and pre-term birth. Our research is elucidating the pivotal role of IGF-II in placental invasion, differentiation and growth. IGF-II promotes trophoblast invasion while TGF β1 inhibits it. We have discovered that IGF-II, under the influence of the low oxygen environment that characterises the first trimester, interacts with the IGF2R and the plasminogen activator system in 7-8 weeks human placental villous explants to promote trophoblast differentiation down the invasive pathway. In addition, treatment of the guinea pig with IGF-I or –II in early to mid pregnancy increases fetal weight near term. IGF-II improves the placental structural capacity for exchange near term, while IGF-I reduces maternal fat deposition, presumably affecting substrate availability. Both IGFs sustainedly improve placental transport of glucose and amino acids. Hence, the type 1 and 2 IGF receptors are likely to mediate different IGF actions during early pregnancy. Gene ablation studies have shown that, later in gestation, IGF-II plays an important role in placental transport functions. Therefore, factors that reduce placental expression of IGF-II are likely to compromise placental exchange late in gestation when the demands of the fetus escalate. We have also discovered that repeated glucocorticoid treatment on days 104, 111 and 118 of pregnancy in the ewe significantly reduces placental IGF-II and IGF2R mRNA expression at day 145 just before term. This
treatment also reduces fetal growth and therefore is likely to impair placental transport functions. Together these data demonstrate that IGF-II is a key player in placental development and function.

008

MACROPHAGE INHIBITORY CYTOKINE-1: ROLES IN TROPHOBLAST FUNCTION AND DECIDUAL PREPARATION

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1Obstetrics and Gynaecology, Monash University, Clayton, VIC, Australia
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3Prince Henry's Institute of Medical Research, Clayton, VIC, Australia

Successful placentalisation is fundamental to the development of a healthy pregnancy and delivery of normal well grown baby. Understanding and manipulating placentalization is therefore key to improving outcomes in various pregnancy disorders such as miscarriage, fetal growth restriction and pre-eclampsia. Over recent years, we have been exploring the roles of macrophage inhibitory cytokine-1 (MIC-1), a transforming growth factor-ss superfamily member, in the regulation of placentation, decidualisation and subsequent pregnancy success. We have shown that MIC-1 is localized to the syncytiotrophoblast layer of the placenta and that MIC-1 production is down regulated in invasive extravillous trophoblast cells. Consistent with this, MIC-1 inhibits the activation of matrix metalloproteinases –2 and –9 in first trimester trophoblast and inhibits outgrowth from villous explants. These data suggest that MIC-1 may regulate trophoblast invasion/placentation. MIC-1 is also localized to the endometrium in both glandular and stromal cells with increasing immunostaining in secretory and decidualised tissues. In vitro, MIC-1 secretion by endometrial stromal cells increases during decidualisation and, in turn, MIC-1 facilitates the process of decidualisation. We have also undertaken a number of clinical studies of MIC-1 levels in maternal serum. In asymptomatic women who subsequently miscarry, first trimester MIC-1 levels are profoundly lower than in women with a subsequently normal pregnancy, consistent with MIC-1 having important roles in early pregnancy establishment. These data offer the potential for new clinical diagnostics and therapeutics. In summary, MIC-1 appears to have a number of potentially important functions in the early human placenta and decidua consistent with physiological roles in normal placentation. Whether these functions are key to successful pregnancy and the diagnostic utility of MIC-1 in early pregnancy remain key questions for our group.

009

INTERLEUKIN 11 AND LEUKEMIA INHIBITORY FACTOR: MECHANISMS AND INTERACTIONS IN IMPLANTATION AND PLACENTATION

E. Dimitriadis
Prince Henry's Institute of Medical Research, Clayton, VIC, Australia

The successful implantation of the human embryo into a receptive endometrium leads to the formation of a functional placenta. Implantation failure results in infertility, while impaired implantation leads to inadequate placentation. Deficiencies in placental development can result in early abortion, or pre-eclampsia and intrauterine growth restriction. Currently there is no way of diagnosing endometrial infertility in women or of establishing whether the placenta is developing adequately. It is critically important to understand the molecular mechanisms of implantation because deficiencies in implantation have such serious consequences. Endometrial interleukin (IL)-11 and leukemia inhibitory factor (LIF) belong to the IL-6 family of cytokines and are two of very few molecules unequivocally required for embryo implantation and establishment of pregnancy in mice. In humans, IL-11 and LIF are produced by the endometrium and placenta in a spacial and temporal pattern suggestive of roles in uterine receptivity, endometrial stromal cell decidualization and trophoblast function. IL-11 advances decidualization of human endometrial stromal cells and LIF enhances endometrial stromal cell survival in vitro. However, roles for IL-11 in endometrial epithelial and trophoblast function are unknown, and for LIF, poorly understood. New evidence will be discussed in terms of the roles and interactions of IL-11 and LIF in uterine receptivity. A key issue in placental development is what controls trophoblast invasion during early placental development. Novel roles for IL-11 and LIF in trophoblast invasion will be presented. Studies that highlight the potential use of IL-11 and LIF as targets of infertility will also be discussed.

010

DETERMINANTS OF TISSUE AND LIGAND SELECTIVITY IN THE MINERALOCORTICOID RECEPTOR

P. J. Fuller, V. Yao, F. M. Rogerson
Prince Henry's Institute of Medical Research, Clayton, VIC, Australia

The mineralocorticoid receptor (MR) differs from the other steroid receptors in that it responds to two physiological ligands, aldosterone and cortisol (1). In epithelial tissues, aldosterone selectivity is determined by the activity of 11β hydroxysteroid dehydrogenase type II. In other tissues, including the heart and regions of the CNS, cortisol is the primary ligand for the MR; in some tissues it may act as an antagonist. Clinical trials demonstrate a benefit of MR antagonists in the treatment of cardiac failure, however this benefit is compromised by hyperkalaemia. There is thus a need to search for tissue and ligand-specific determinants of MR activation.
Using a chimeric approach (2), we exploited the inability of the GR to bind aldosterone to identify the region of the MR ligand-binding domain (LBD) that confers aldosterone binding. We have narrowed this to a region of 25 amino acids, curiously the residues in this region that permit aldosterone binding do not contribute to the ligand-binding pocket.

Although the steroid receptors are modular, interactions may occur between domains. The N/C-interaction (3) is aldosterone-dependent but unexpectedly cortisol is an antagonist.

Nuclear receptor mediated transactivation is critically dependent on, and modulated by, co-regulatory molecules. A yeast-2-hybrid kidney cDNA library screen with the MR LBD has identified proteins which interact with one but not both MR ligands.

Further characterisation of these interactions may provide the basis of screens for the identification of “selective mineralocorticoid receptor modulators”.

(3) Fuller PJ and Young MJ. Hypertension 46:1227, 2005.

011

ROLES OF ORPHAN RECEPTOR LRH-1 IN REPRODUCTION AND CANCER

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Liver Receptor Homologue-1 (LRH-1) is an orphan member of the nuclear receptor superfamily that belongs to the NR5A subgroup of receptors. Originally identified as a liver-specific factor that regulates expression of alphafetoprotein, LRH-1 has now been implicated in a variety of processes including cholesterol and bile acid synthesis, steroidogenesis and embryonic development. We have shown roles for LRH-1 in regulating aromatase expression in adipose tissue, testis and granulosa cells. LRH-1 also appears to mediate the over-expression of aromatase that occurs in adipose tissue of breast cancer patients, thereby providing the source of oestrogens for growth of postmenopausal ER+ tumours. In addition, LRH-1 directly stimulates proliferation of breast cancer epithelial cells by stimulating expression of G1 cyclins. As such, it is an attractive target for drug development. As an orphan receptor, however, LRH-1 is constitutively active in the absence of ligand, and to date no antagonists have been identified. We are using complementary approaches to identify selective LRH-1 modulators. Firstly, by using phage display we have isolated small peptides that can inhibit LRH-1 activity by preventing its ability to interact with endogenous co-activators. Secondly, in silico approaches are being utilised to identify small “drug-like” molecules that either mimic the peptide antagonists by binding to the same site, or else occupy the classical ligand binding site of the receptor. These approaches may produce useful tools to dissect the functions of LRH-1 in endocrine tissues, and also have the potential to realise a new class of nuclear receptor modulators.

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VITAMIN D-WNT PATHWAY INTERACTIONS IN SKELETAL AND NON-SKELETAL CELLS

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Active hormonal vitamin D, calcitriol, inhibits cell proliferation and induces differentiation and apoptosis in normal and tumor cells. Wat signaling is involved in embryonic developmental and in adult tissue homeostasis, regulating cell fate specification, proliferation and differentiation. In addition, Wnt pathway dysregulation occurs in tumor cells. Secreted Wnt family glycoproteins act through Frizzled receptors to stimulate canonical β-catenin-mediated transcriptional responses. Evidence that human bone mass is strongly affected by mutations of LRP5, a Frizzled co-receptor, has led to investigation of interactions between Wnt and other bone regulatory pathways, including the vitamin D response pathway. In addition, study of vitamin D-Wnt pathway interactions has also been stimulated by findings that vitamin D analogues promote differentiation of human colon carcinoma cells, in which β-catenin protein level or activity is often elevated. Calcitriol-bound vitamin D receptor (VDR) can directly inhibit the Wnt response by interaction with β-catenin, sequestering it away from the Wnt-responsive TCF transcription factor complex. As a result, activation of vitamin D responsive promoters is potentiated while transcriptional regulation of Wnt target genes is reduced. These divergent transcriptional effects are due to direct interaction between the β-catenin C-terminus and the VDR activation function-2 domain, with acetylation at the β-catenin C terminus differentially regulating the transcriptional responses1. Calcitriol can also indirectly affect canonical Wnt pathway activity in osteoblasts by enhancing LRP5 gene transcription2 and in colon carcinoma cells by stimulating expression of E-cadherin, which depletes nuclear β-catenin by promoting its accumulation at the plasma membrane3. The relative significance of these direct and indirect interaction mechanisms appears to be dependent at least in part upon cell type and the level of β-catenin present.

(2) Fretz et al. 2006. Molec Endocrinology e-published.
NUCLEAR RECEPTORS IN METABOLISM: THE SKI PHENOTYPES AND THE NORPHANS
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Nuclear Receptors (NR) are ligand-activated transcription factors that play key roles in growth, development and metabolism. The NR gene superfamily comprise the steroid receptors (estrogen, androgen, glucocorticoids amongst others), retinoid x-receptor (RXR) heterodimers (including thyroid, retinoic acid, and PPARs) and a large orphan receptor sub-family of receptors with no known ligands (NOR-1/Nur77, COUPTF, LXR and many others). NRs expressed in skeletal muscle, fat and liver act as nutritional sensors to regulate metabolic target gene transcription to maintain energy homeostasis through direct effects on lipid, glucose and energy metabolism. As such, NRs involved in regulation of metabolism are primary targets for pharmacotherapeutic intervention to prevent and treat diabetes, cardiovascular disease and the metabolic syndrome. NRs being essential for normal growth and development interact with many other signalling pathways, including the transforming growth-factor- β (TGF-β) pathway, to amplify the diversity and complexity of their physiological control on growth. The Ski proto-oncogene, a negative regulator of the TGF- β signalling, recruits corepressors or prevents coactivator recruitment to the active transcriptional complex to determine the ultimate transcriptional outcome and the anti-proliferative potential. Ski in vivo and in vitro modulates skeletal muscle metabolism in part through an interaction and via regulation of NR dependent effects on lipogenesis and metabolism. In this seminar, the functional role of several orphan nuclear receptors in skeletal muscle metabolism will be presented, in addition to the role of Ski crosstalk with the NR signalling pathway involved in development of the metabolic syndrome.

ASPECTS IN THE DIAGNOSIS AND MANAGEMENT OF PROLACTINOMA
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Human prolactin was isolated in the 1970's and it was soon recognised that hyperprolactinaemia resulted in a syndrome of amenorrhoea/galactorrhoea. Subsequently, it has been shown that hyperprolactinaemia may be the cause of secondary amenorrhoea in up to one third of cases. Prolactinomas are the commonest form of pituitary adenoma, and make up approximately 30% of all pituitary neoplasms. The basic principles of investigation involve excluding physiological and non-neoplastic causes of hyperprolactinaemia, pituitary neuroimaging and biochemical assessment of pituitary function. The recognised indications for treating hyperprolactinaemia include hypogonadism (oligo-amenorrhoea in women, androgen deficiency in men), significant symptomatic galactorrhoea and tumour mass effect, particularly where visual pathways are compromised. Where hyperprolactinaemia is asymptomatic, no specific treatment other than periodic observation may be required. Once a prolactinoma is diagnosed, the usual first line of treatment is with a dopamine agonist. Currently, the dopamine 2 receptor specific agonist Cabergoline is the most widely used agent in clinical practice. Delivered once or twice weekly, it normalises prolactin levels in over 90% of subjects with pathological hyperprolactinaemia. The usual dose range is from 0.5 to 3mg per week, but higher doses may be used in resistant cases. Published data regarding macroadenoma shrinkage are uncontrolled but also demonstrate equal or superior efficacy compared to older studies of Bromocriptine, and occurs in approximately 80% of patients. Recent evidence suggests that over 60% of cases treated for 3-4 years with Cabergoline may enter a long term remission on cessation of the drug. Resolution of the adenoma on MRI is predictive of remission. Surgery is generally reserved for a) dopamine-agonist resistant tumours, b) adverse effects of dopamine agonists or c) where it is desirable to obtain a histological diagnosis. Management of prolactinoma during pregnancy will be briefly discussed. Historically, Bromocriptine has been preferred although there is no evidence that Cabergoline is associated with an increased rate of foetal anomalies.

ACROMEGALY: NEW FRONTIERS IN MANAGEMENT
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Over the last few decades, there have been important advances in the fields of neuroendocrinology, cell biology, clinical chemistry, drug development, imaging, neurosurgery and radiotherapy, all of which have had a major impact in the management of acromegaly. The merits of growth hormone (GH) and insulin-like growth factor (IGF)-1 measurements in the diagnosis and in the assessment of therapeutic outcomes of acromegaly have been intensively studied. The biochemical targets for treatment are a growth hormone of <2.5 ng/mL and a normal, age-adjusted insulin-like growth factor-1. Until 20 years ago, dopamine agonists were the only class of pharmaceutical agents available to control acromegaly. They have a limited adjunctive role, even with the development of second-generation selective agonists such as cabergoline. Surgery and radiotherapy were the mainstay of acromegaly management before the advent of the effective pharmacological therapies of the
modern era: somatostatin analogues and pegvisomant, a growth hormone receptor antagonist. Somatostatin analogues achieve biochemical control in approximately 60% of patients. Pegvisomant, which is available in the USA and Europe and has just been registered in Australia, normalizes IGF-1 in nearly all patients but has no effect on tumour mass. Surgery is an appropriate first-line therapy for microadenomas as the chance of success is high. For large and/or invasive tumours where the prospect of surgical cure is remote, first-line therapy is somatostatin analogue treatment with debulking surgery having an adjunctive role to achieve tight control or to alleviate compression of the optic chiasm. Although acromegaly remains a challenging disease to manage, the expanding range of therapeutic options is likely to result in a better outcome for patients and offers the potential to tailor therapy based on a patient's individual requirements.

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INSIGHTS INTO MONITORING THERAPY, AND CHALLENGES IN MANAGEMENT OF HYPOPITUITARY PATIENTS
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Pituitary hormone secretion is complex, dynamic and responsive to multiple external stimuli. This makes replication of normal hormone function in the hypopituitary patient very challenging. Evaluation of possible deficiencies of pituitary hormone secretion is the first challenge the clinician must face. The utility of different static and dynamic tests will be discussed. Replacement therapy with thyroid hormones and gonadal steroids are relatively straightforward, although the route of administration and dosage of sex hormones need to be optimized for each individual. More problematic is determination of the appropriate dosage and formulation for glucocorticoid replacement. We lack any effective method for measuring glucocorticoid action on target tissues, and measurements of circulating adrenal steroids are complicated by diurnal rhythms and variation in binding proteins. Possible approaches to monitoring of cortisol levels and action in ambulatory patients will be discussed. Even greater controversy surrounds the issues of Growth Hormone replacement in GH deficient adults, and androgen therapy in women with primary or secondary hypoadrenalism. Available evidence will be reviewed and balanced with practical aspects of cost and availability of appropriate therapeutic agents in Australia. Finally, some difficulties in management of diabetes insipidus will also be discussed. Patients with hypopituitarism have complex problems, and many remain symptomatic despite apparent “normalization” of their hormone concentrations. A flexible and individualized approach is important in achieving the best possible outcome for each patient.

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NUTRACEUTICAL AND PHARMACEUTICAL EFFECTS ON UTERINE AND HORMONAL RESPONSES ASSOCIATED WITH EARLY PREGNANCY IN LACTATING DAIRY CATTLE
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A timed insemination program was used to investigate mechanisms through which polyunsaturated fatty acids (PUFA) and bovine somatotropin (bST) may increase fertility. Cows were assigned randomly to be inseminated (d 0) or not inseminated, and to receive 0 or 500 mg of bST (at d 0 and d 11) (i.e., C [cyclic], bST-C, P [pregnant], bST-P). Furthermore, a fish oil-enriched lipid supplement (FO; 1.9% of dietary DM initiated at 10 DIM) was evaluated in cyclic cows with (bST-FO; bST-C) and without (FO; C) bST. On d 17 (~ 94 d DIM) cows were slaughtered, uteri flushed and endometrial tissue collected. BST increased milk production, pregnancy rate (83% [5/6] > 40% [4/10]), conceptus length (45 > 34 cm) and interferon-γ in uterine luminal flushings (9.4 > 5.3 mg) with no effect on interferon-γ mRNA concentration in the conceptus. Feeding FO to cyclic cows increased proportions of eicosapentaenoic and docosahexaenoic acids while reducing the proportion of arachidonic acid in the endometrium. Cyclic cows fed FO had lower plasma insulin than control-fed cyclic cows, and FO altered plasma GH (bST-FO > bST-C) and IGF-I (bST-C > bST-FO) responses to bST. Endometrial IGF-I mRNA was reduced in pregnant cows. IGF-II mRNA was increased in the endometrium of P and bST-treated cows fed the control diet. Cows fed FO had increased concentrations of IGF-II mRNA when bST was not injected. IGFBP-2 mRNA was increased in bST-P cows, whereas bST decreased IGFBP-2 mRNA in all cyclic cows. FO decreased FGF-2 and increased progesterone receptor (PR) mRNAs. BST increased PR mRNA in endometrium of C but not in FO-fed or P cows. Concentrations of ERα mRNA and protein, and oxytocin receptor mRNA were decreased in P compared to C cows. Immunohistochemistry indicated that P and FO decreased ERα abundance in luminal epithelium. PGH-2 protein was elevated in P cows and localized to the luminal epithelium. Both FO and bST treatments reduced staining intensity of PGHS-2 protein. In summary, pregnancy and bST altered endometrial gene expression. Cyclic cows responded differently to bST than pregnant cows. Feeding FO modulated responses in a manner that may favor maintenance of pregnancy.
IODINE SUFFICIENCY ACROSS AUSTRALIA: DO WE CURRENTLY MAKE THE GRADE?
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Over the past 7 years several localised, regional studies in South Eastern Australia and Tasmania have documented the re-emergence of mild to moderate iodine deficiency in adults and children. To provide a comprehensive snapshot of iodine nutrition throughout Australia we undertook a National Iodine Nutrition Study between mid 2003 and end 2004.

Design and Setting:
The survey was a cross-sectional study of 8 to 10 year old school children, randomly selected from government and non-government primary schools, in the mainland Australian states of New South Wales, Victoria, South Australia, Western Australia and Queensland. The sample consisted of 1,709 students from 88 schools, comprising 881 boys and 828 girls. 1) Urinary iodine excretion levels (UIE) were determined and compared with WHO/ICCIDD criteria for the severity of iodine deficiency. 2) Thyroid volumes measured by ultrasound were compared with new international reference values (WHO/ICCIDD).

Results:
On a State basis, NSW and Victorian children are mildly iodine deficient with median UIE levels of 89 μg/L and 73.5 μg/L, respectively. South Australian children are borderline iodine deficient with a median UIE of 101 μg/L. Both Queensland and Western Australian children are iodine sufficient with median UIE levels of 136.5 μg/L and 142.5 μg/L, respectively. Ongoing studies in NSW of iodine nutrition in pregnant women, and their offspring, confirm mild to moderate iodine deficiency is widespread throughout the State.

Conclusion:
The results of this study confirm the existence of inadequate iodine intake in the Australian population and call for the implementation of mandatory iodisation of all edible salt in Australia. In the interim, we recommend iodine supplementation be considered for pregnant women, those contemplating a pregnancy, and breastfeeding mothers.

SEARCHING FOR STEM CELLS IN THE KIDNEY
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The kidney, unlike the liver, never undergoes a structural repair or regenerative process in response to damage. Our understanding of kidney development suggests that nephron endowment is finalized prior to birth and that while they can hypertrophy, no new nephrons are ‘born’ after birth. However, recent data, particularly in the brain but also in other postnatal tissues including the heart and adipose, suggests that there may be stem cells present in adult organs previously regarded as non-proliferative during the perinatal period. We have taken several approaches to investigate the possibility of adult stem cells in the murine kidney. The first involved defining markers of renal progenitors by examining the expression profile, multipotency and renal lineage potential of a putative stem cell population in the kidney, the Hoechst effluxing side population. In this presentation, we will discuss the results of these studies and reflect on what implications these might have in the development of novel treatments for renal failure.

IDENTIFICATION OF MAMMARY STEM CELLS AND THEIR ROLE IN BREAST CANCER
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The identity and purification of mammary stem cells (MaSCs) has proved elusive due to the lack of defined markers. However, we have recently isolated discrete populations of mouse mammary cells on the basis of cell-surface markers and identified a subpopulation (Lin-C2D29hiCD24+) that is highly enriched for MaSCs as assayed by in vivo transplantation. Indeed, we demonstrated that a single cell, marked with a lacZ transgene, could reconstitute a complete mammary gland in vivo. The transplanted cell contributed to all three mammary epithelial lineages and in extensive lobuloalveolar units were generated during pregnancy. Serial transplantation revealed that these cells have self-renewing capacity. These data establish that single cells within the Lin-C2D29hiCD24+ population have the multipotent and self-renewing properties that define the MaSC. To further characterise the different mammary epithelial populations, we have investigated the expression of important prognostic markers of human breast cancer, including the estrogen receptor α (ERα), and progesterone receptor (PR), and provide
evidence for differential expression amongst the various subsets. Finally, we show that the stem cell population was expanded in premalignant mammary tissue from MMTV-wnt-1 but not MMTV-neu transgenic mice, indicating that stem cells are the likely tumour-initiating cells the wnt-1 model of breast tumorigenesis.

GERMLINE STEM CELLS IN THE OVARY

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The notion of a fixed, non-renewable pool of oocytes in the ovary around birth (1) has been questioned (2,3,4). The presence of germline stem cells (GSC) giving rise to new oocytes in the adult ovary has been proposed (3,4) but challenged on theoretical and methodological grounds (5,6). Two reports provide new data that inform this debate (2,6). Byskov et al (6) injected 30-day old C57BL/6 mice with BrdU and could not find labeled oocytes in primordial or later stage follicles 8 days later. This argues against the presence of mitotically-active GSC in the mouse ovary, but does not exclude differentiated GSC arising from within the ovary or from external sources. We quantified all healthy follicles in C57BL/6 mouse ovaries between days 1 and 200 (n=6-10) using unbiased stereological methods (2). Mean numbers of healthy follicles fell 60% between days 1 and 7, primarily due to expulsion from the ovary. Although we saw no evidence for GSC, rare mitotic figures in unidentified cells were noted between days 1 and 12. From day 7 to 100 mean numbers of primordial or total follicles per ovary were not significantly depleted, but declined by day 200 to about 10% of day 1 levels. Our data supports the hypothesis of follicle renewal in postnatal and adult ovaries of C57BL/6 mice by an as yet unknown mechanism.

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ADULT STEM/PROGENITOR CELLS IN THE ENDOMETRIUM

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The human endometrium undergoes regeneration, differentiation and regression with each menstrual cycle, following parturition and in post-menopausal women taking estrogen replacement therapy. In other regenerative tissues, rare populations of adult stem cells have been identified. While it was postulated many years ago that endometrial stem/progenitor cells were responsible for endometrial regeneration, it was not until recently that we provided the first evidence for their existence in human and mouse endometrium, setting a new paradigm in uterine biology (1,2). We demonstrated that 0.22% of endometrial epithelial cells and 1.25% of stromal cells were clonogenic, each producing two morphologically distinct colony types (1). The large clones (colony forming units, CFU) of small, densely packed cells were rare (0.09% of epithelial and 0.02% of stromal), whereas large epithelial and stromal CFU exhibited several adult stem cell properties; high proliferative potential and self-renewal. Large stromal CFU cultured in appropriate induction media also underwent multilineage differentiation into mesenchymal lineages; fat, smooth muscle, bone and cartilage. Bone marrow mesenchymal stem cell (MSC) phenotypic markers CD29, CD44, CD90, CD73, CD105, CD146 were expressed by large epithelial-like cells at the endometrial-myometrial junction (2). These rare epithelial and stromal LRC rapidly proliferated on estrogen-induced endometrial regeneration, despite lack of estrogen receptor-α expression. Our data suggest that rare populations of epithelial progenitors and MSC-like cells exist in human and mouse endometrium and may be responsible for initiating endometrial regeneration.

(2) Chan RWS, Gargett CE (2006) Stem Cells, 24 (in press)
NEW GENES THAT CONTROL REPRODUCTION IN THE HUMAN AND THEIR GENOTYPE-PHENOTYPES: EVIDENCE FROM HUMAN DISEASE MODELS
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In all mammalian species, gonadotropin-releasing hormone (GnRH) represents the key interface between the way that the CNS views the outside world and how it transmits signals internally to its reproductive endocrine milieu. Since it is the first and initiating hormone in a complex reproductive cascade that involves gonadotropin biosynthesis and secretion, gonadal steroidogenesis and germ cell maturation, and behaviour, it can be viewed as the "pilot light of reproduction". Therefore, understanding the genes and signals that modulate the developmental fate and secretory actions of GnRH neurons in man remains a major question as defined in Science Magazine's 125 Outstanding Scientific Questions in 2005. To gain insight into this problem, we have used the human disease models of normosmic idiopathic hypogonadotropic hypogonadism (nIHH) and Kallmann Syndrome (KS) to elucidate the genes that control GnRH's secretion and action. Since patients with these conditions represent isolated defects in the secretion or action of GnRH, understanding their genetic basis has proven to be an important avenue of biologic insights into this problem. This lecture will report on several new genes that are responsible for control of this key reproductive peptide in the human that we have identified using these human disease models, including GPR54, Metastin, FGFR1, and GnRHR. It will review new mutations in each and their genotype/phenotype correlations as well as useful clinical points for their counseling and management.

KISSPEPTIN ACTIVATION OF GNRH NEURONS TO INITIATE PUBERTY AND OVULATION.
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Evidence is accumulating for a critical role of kisspeptin signaling within the neural circuitry controlling fertility. Our studies have focussed upon understanding the cellular mechanisms through which kisspeptin controls the activity of the gonadotropin-releasing hormone (GnRH) neurons in mice. Immunocytochemical approaches have demonstrated that a large population of kisspeptin neurons located in the rostral periventricular region of the hypothalamus develop just prior to puberty. Studies using a Cre-dependent Pseudorabies virus retrograde tracing technique in transgenic GnRH-Cre mice have shown that a sub-population of periventricular kisspeptin neurons project directly to GnRH neurons. Across development, GnRH neurons can be seen to receive kisspeptin-immunoreactive fibre appositions from postnatal day 25 onwards and electrophysiological studies in GnRH-GFP mice show that the percentage of GnRH neurons responding directly to kisspeptin increases across puberty. These studies suggest that periventricular kisspeptin neurons innervate and activate GnRH neurons directly to help bring about puberty. Estrogen positive feedback initiates ovulation through a neural pathway that involves sexually dimorphic, estrogen receptor alpha (ERα)-expressing neurons that activate GnRH neurons. Our recent studies have shown that periventricular kisspeptin neurons in the mouse (1) are sexually differentiated with females having 10-fold greater numbers of kisspeptin neurons compared with males, (2) express ERα, and (3) are activated by estrogen positive feedback using estrogen or ERα-selective agonists. Viral retrograde tracing shows that ERα-expressing kisspeptin neurons are primary afferents to GnRH neurons. Coupled with electrophysiological evidence for a massive and prolonged excitatory action of kisspeptin on GnRH neurons at proestrus, these data strongly suggest that periventricular kisspeptin neurons are a key component of the neural mechanism initiating ovulation.

THE BIOLOGY OF KISSPEPTINS AND GPR54
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In July 2005, Science magazine listed as one of its 125 greatest unanswered scientific questions "What controls puberty”? The power of genetics resulted in the discovery of the GPR54/Metastin system in the human by 2 groups in 2003 (DeRoux et al; Seminara et al). Since then, it has become clear that this system, previously completely overlooked by basic investigators attempting to determine the long-elusive "puberty gene”, actually fulfills the criteria for a ligand receptor system that serves as a major gatekeeper of puberty and sexual maturation in several species including the human, mouse, and monkey. GPR54 receptors are located in the medial basal hypothalamus with >75% of GFP labelled GnRH neurons demonstrating their presence. GPR54 levels increase at puberty in both sexes in rodents and monkey. Their neuroanatomic localization in the arcuate nucleus and the AVPV, both important and known sites for the neuroendocrine control of GnRH secretion, position them well to be major regulators of GnRH secretion during puberty. GPR54 levels also appear to be sex steroid responsive although our understanding of the transcriptional control of GPR54 is still in its infancy. The precursor 145 aa peptide ligand for GPR54, Kisspeptin, is cleaved at a dibasic cleavage site and gives rise to an amidated 54 aa carboxy terminal fragment.
termed Metastin because of its previously described role in limited metastatic disease in several malignant cell lines. It appears that all biologic activity resides in the amidated carboxyterminal decapptide sequence of Kisspeptin/Metastin although the circulating form of this precursor molecule is not yet clear. Metastin has been determined to be the most sensitive stimulator of GnRH-induced LH secretion yet discovered with levels as low as pM being able to stimulate GnRH secretion in rodents and primates. Metastin administration also causes GnRH antagonist-blockade of LH release (indicating the essential role of GnRH secretion and action in the ensuing LH release). Circulating levels of metastin are detectable in the human and increase 10,000 fold during pregnancy in a pattern that is quite different from hCG. This lecture will focus upon this sytem and its relationship with GnRH secretion.

**027**

**KISSPEPTIN AND GPR54: MOLECULAR CONDUITS FOR PUBERTY ONSET AND CENTRAL INTEGRATION OF ENERGY BALANCE AND REPRODUCTION**

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Based on the observation that inactivation of the G protein-coupled receptor GPR54 is linked to hypogonadotropism in humans and mice, the essential role of this receptor and its putative ligands (kisspeptins, encoded by KiSS-1 gene) in the control of reproduction was first proposed in late 2003. Indeed, such a contention has now been fully substantiated by a number of genetic, molecular, physiologic and pharmacological studies. We will review herein the available evidence for the key role of KiSS-1/GPR54 system in the timing of puberty and signaling of energy balance and metabolic information onto the centers governing reproductive function. Concerning puberty onset, hypothalamic expression of KiSS-1 and GPR54 genes has been proven developmentally regulated, with maximum levels at puberty in rodents and primates. Moreover, functional studies have disclosed that enhanced KiSS-1 function (through increased kisspeptin tone and signaling efficiency) takes place at the time of puberty. Nonetheless, pubertal activation of KiSS-1 system appears to be exquisitely modulated, as excessive enhancement of KiSS-1 tone at puberty evokes the ‘paradoxical’ suppression of the gonadotropic axis. Regarding integration of energy status and reproduction, functional and expression analyses have demonstrated that situations of negative energy balance, linked to hypogonadotropism, decrease the hypothalamic expression of KiSS-1 gene, while exogenous kisspeptin is able to normalize acute gonadotropin responses and restore pubertal activation of the reproductive axis in undernutrition. Similar observations have been obtained in models of altered metabolism and gonadotropin secretion, such as experimental diabetes, where decreased KiSS-1 expression was associated to defective leptin levels, in line with recent data showing reduced KiSS-1 mRNA levels in ob/ob mice. Altogether, these data evidence that, among other essential roles in reproduction, the hypothalamic KiSS-1/GPR54 system operates as pivotal molecular conduit in the timing of puberty onset and for relaying metabolic information onto the centers governing the gonadotropic axis.

**028**

**THE ROLE OF KISSPEPTIN IN MEDIATING SEX STEROID FEEDBACK CONTROL OF GNRH**  
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The Kiss1 gene encodes a family of peptides called kisspeptins. These peptides are the endogenous ligands for the G protein-coupled receptor, GPR54, and play a vital role in the regulation of GnRH and in turn gonadotrophin secretion. In many species, centrally administered kisspeptin stimulates gonadotrophin secretion in a GnRH dependent manner. Moreover, virtually all GnRH neurons co-express GPR54. In the hypothalamus, the vast majority of kisspeptin producing cells (those expressing KiSS-1 mRNA) also express sex steroid receptors, particularly oestrogen receptor alpha. Thus, sex steroids are able to directly regulate the expression of KiSS-1 mRNA, implicating kisspeptin as a link between sex steroids and GnRH feedback. In the arcuate nucleus (Arc) of the rodent, sex steroids inhibit the expression of KiSS-1 mRNA, suggesting that the kisspeptin secreting neurons here are the conduit for the negative feedback regulation of GnRH/gonadotrophin secretion. However, in the anteroventral periventricular nucleus (AVPV), sex steroids induce the expression of KiSS-1 mRNA, implying that these kisspeptin neurons play a role in the positive feedback regulation of GnRH/gonadotrophin secretion. Thus, it is conceivable that kisspeptin neurons in the AVPV are central processors for generating the preovulatory luteinising hormone surge in the female.

**029**

**LABORATORY MEASURES IN CLINICAL ENDOCRINE PRACTICE: BONE MARKERS**  
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Current investigations for patients with osteoporosis focus on assessment of bone mass with densitometry. However, clinicians commonly order laboratory tests to identify secondary causes of osteoporosis as well as monitor response to therapeutic interventions. The use of bone markers has been extensively studied and these markers reflect generalised skeletal
remodelling. Bone markers may thus offer diagnostic utility, prognostic information and represent a useful tool for therapeutic monitoring. Bone markers are classified as either bone formation or bone resorption markers depending on which remodelling process they mainly represent. In most instances both remodelling processes are balanced and either bone marker will reflect the degree of bone remodelling activity. However, because bone resorption is shorter than formation, resorption markers respond faster to changes in remodelling than formation markers. A number of cases will be presented that highlight the current utility as well as limitations of bone markers in clinical practice. Specifically, a thorough understanding of preanalytical factors, analytical issues including lack of standardisation and postanalytical interpretation of results in the context of pathological skeletal disorders will be discussed. All of these issues can result in significant variation in results and an understanding is required when interpreting individual bone marker responses. Bone markers have a limited role in diagnostic stratification, however could have a greater role in prognostic stratification and therapeutic monitoring which may result in routine clinical application. Recent evidence has indicated that pretreatment bone turnover may predict the reduction in nonspine fractures with alendronate therapy. Reduction in bone turnover occurs earlier than bone density changes following risedronate therapy and accounts for more than 50% of the predicted fracture reduction with treatment. Lastly bone markers may have a role in defining persistence with therapy and compliance with dosing recommendations.

MACROPROLACTIN

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Diagnosing hyperprolactinemia has always been confounded by the possible presence of macroprolactin which has been dubbed “false hyperprolactinemia”. If not properly investigated macroprolactin can lead to diagnostic confusion, invasive and unnecessary investigations. Prolactin can be present in the circulation as the monomer alone (molecular weight (MW) 23,000), or as a variable mixture of monomer, a polymeric complex of between 40 and 60,000 MW and the monomer bound to an immunoglobulin (MW 150-170,000). Both the monomer and the polymeric complex known as big prolactin have been reported to be biologically active. The immunoglobulin bound form is described as big big prolactin (macroprolactin), which has a long half life in the circulation and limited bioactivity. It is important to screen high prolactin levels for the presence of macroprolactin before commencing an investigation for an adenoma. Some prolactin assays do not measure macroprolactin and others show variable ability to determine its presence. Routine screening of high prolactin levels has to be done with an assay that detects macroprolactin and has been confirmed to be able to measure Prolactin in the presence of PEG. The immunoglobulin bound prolactin is precipitated with 12.5% PEG (w/v) and a recovery of 40% or less of the immunoreactive prolactin in the supernatant was indicative of the presence of macroprolactin. This also can be misleading and reporting only the PEG recovery can lead to some confusion. The presence of a microadenoma cannot be excluded, unless the recovered prolactin levels returned to the normal range after treatment. To add further confusion using gel exclusion chromatography we have found patients with big prolactin levels that would have appeared to be macroprolactinemic if only the PEG screening assay procedure was used. Many of the confusing patients may be being detected earlier now because of the increased awareness of macroprolactin in hyperprolactinemia. It is important to recognize the limitations of the screening assay as well as the ability of different prolactin assays to detect macroprolactinemia. It is concerning us that we are detecting an increasing number of patients with all three forms of prolactin in the circulation and a number of these may be incorrectly attributed to the presence of macroprolactinemia.

NUCLEAR RECEPTOR COREGULATORS – GETTING TO THE HEART OF HORMONE ACTION

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Discovery of the nuclear receptor coregulators, exemplified by SRC-1, has revolutionized our understanding of hormone action, and provided an opportunity to develop new diagnostic and prognostic markers as well as potential new therapeutics for a variety of human diseases. Numerous coregulators (comprising coactivators and corepressors) have been identified in the past few years, and the challenge is to define their molecular mechanisms of action, their functional role in a tissue- and disease-specific manner and translate these findings into meaningful clinical outcomes. In the past few years, as part of our studies to understand signaling pathways and identify novel targets in hormone-dependent cancers, we identified several novel nuclear receptor coregulators that bind a specific RNA coregulator (SRA, Steroid receptor RNA Activator) which is aberrantly expressed in human breast cancer (BCa). These SRA-binding coregulators contain distinct RNA-binding domains from two different structural families, and each modifies SRA-mediated coregulation of multiple nuclear receptor signaling pathways, including estrogen, androgen, glucocorticoid and thyroid. For example, SLIRP is a novel protein that corepresses the estrogen, thyroid and vitamin D nuclear receptor signaling pathways, interacts with multiple other coregulators, including SHARP, SKIP and NCoR, and augments the effects of tamoxifen. Remarkably, the majority of SLIRP resides in the mitochondrion, and it is most highly expressed in the energy-rich tissues (heart and skeletal muscle). Furthermore, SLIRP represses PPARδ signaling suggesting an important role in energy homeostasis and metabolism in heart and skeletal muscle. Taken together, our studies provide insight into the important contribution of SRA-protein interactions to nuclear receptor transcription, support a key physiological role for each of these SRA-binding coregulators in a wide range of nuclear receptor signaling, especially
estrogen signaling in BCa and suggest mechanisms by which aberrant expression could modulate anti-estrogen therapies. Furthermore, they illustrate the bifunctional nature of some coregulators, and for SLIRP, suggest key roles in both hormone-dependent cancer and energy homeostasis signaling pathways.

034
INTRACELLULAR STRESS, MITOCHONDRIAL DYSFUNCTION AND DIABETES COMPLICATIONS
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Intracellular stresses, such as oxidative stress and endoplasmic reticulum (ER) stress, are involved in the development of various diseases. In this lecture, our studies about the impacts of ER stress and reactive oxygen species derived from mitochondria (mtROS) on diabetes and its complications will be presented. The ER plays important functions essential to cell survival. Various conditions that interfere with ER function are called ER stress. Severe ER stress leads to apoptosis through induction of ER stress-associated apoptosis factor CHOP. The Akita mouse with a mutation (Cys96Tyr) in the insulin 2 gene develops diabetes with a reduced beta-cell mass. Overexpression of the mutant insulin in MIN6 cells induced CHOP expression and apoptosis. Targeted disruption of the CHOP reduced islet cell apoptosis and delayed the onset of diabetes in Akita mice. In addition, db/db mice displayed an increase of CHOP and other ER stress-related genes suggesting the involvement of ER stress in the progression of diabetes. The mtROS may play primary role in the development of diabetic complications. In MIN6 cells, hyperglycemia increased mtROS production, and the treatment of the beta-cells with H2O2 suppressed the first phase of glucose-induced insulin secretion. On the other hand, in hepatoma Huh7 cells, mtROS decreased tyrosine phosphorylation of IRS-1, an important insulin signal, via activation of ASK-1-JNK pathway. Therefore, mtROS could prevent insulin secretion and action, which may explain glucotoxicity in diabetes. To further study the role of mtROS in diabetic complications, we created a transgenic (eMnSOD-Tg) mouse that overexpresses MnSOD in endothelial cells. Expression of VEGF and fibronectin mRNAs in retinas was observed in STZ-induced diabetic WT mice, which was completely prevented in diabetic eMnSOD-Tg mice. In addition, the increase of 8-OHdG, a marker of oxidative stress was also suppressed in diabetic eMnSOD-Tg mice. In the relative hypoxia-induced in vivo retinopathy model, retinal flat-mount pictures showed typical central avascular areas in WT mice, which were reduced in eMnSOD-Tg mice. Therefore, normalizing hyperglycemia-induced mtROS could prevent diabetic complications in vivo. Our results indicate that intracellular stresses could be novel targets for prevention and treatment of diabetes and its complications.

035
NOVEL APPROACHES FOR THE TREATMENT OF DIABETES - WEARABLE ARTIFICIAL ENDOCRINE PANCREAS (AEP) AND MILD ELECTRIC AND THERMO GENERATOR (MET)
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Diabetic treatment is sometimes difficult in cases of insulin deficient status and of severe insulin resistance. To conquer these, our group has created a wearable artificial endocrine pancreas (AEP) and an instrument, named MET (mild electric current and thermo generator), which reduces insulin resistance in vivo by mild hyperthermia and electric current. AEP is a closed-loop system with glucose sensor, insulin infusion algorithm, and infusion pump to establish strict glycemic control. To establish the ideal insulin delivery route for AEP, we examined the effectiveness of portal and intraperitoneal insulin delivery routes. The closed-loop portal insulin delivery was feasible with regard to both insulin profiles and hepatic glucose handling in vivo. On the other hand, intraperitoneal route is less invasive and ~70% of infused-insulin could flow into portal vein, and achieved better glycemic control when compared with subcutaneous infusion. Therefore, the portal vein may be the most ideal insulin delivery route but intraperitoneal route could also be beneficial for glycemic control by AEP. Hyperthermia is known to reduce insulin resistance, at least in part through expression of HSP72. We recently found that combination of mild hyperthermia and weak electrical current could efficiently induce HSP72 in culture cells. Therefore, an instrument that can induce mild hyperthermia and weak electrical current in vivo, named MET, was created and applied for model mice of insulin resistance. Treatment of the high fat fed mice with MET twice a week for 8 weeks significantly reduced subcutaneous and visceral fat, reduced fasting insulin level, increased adiponectin level and improved glucose tolerance when compared with sham-treated mice. Fatty liver was also dramatically improved. The HSP72 induction was confirmed in tissues of MET treated mice, which paralleled with improved insulin signaling and increased insulin-stimulated GLUT4 translocation. Therefore, MET could be used for the treatment of patients with type 2 diabetes and/or metabolic syndrome.

036
INHALED INSULIN IN DIABETES TREATMENT
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The alveolar membrane is thin, permeable and has a large surface area. Unlike the gastrointestinal tract, it is relatively free of proteases. It therefore represents a suitable site for insulin delivery provided the hormone can be deposited in sufficient local
INSULIN DELIVERY BY GENETIC ENGINEERING

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Type I diabetes is caused by the autoimmune destruction of the pancreatic beta cells. The only treatment for the disease is the injection of insulin to regulate blood glucose. Despite the best glucose-monitoring procedures the chronic complications of diabetes still develop: retinopathy, neuropathy, nephropathy and macrovascular complications. The only “cure” for diabetes is the transplantation of donor pancreatic tissue, but this is limited by lack of donors and the fact that patients must be immunosuppressed. Ultimately, other cures may come from xenotransplantation, generation of beta cells from human embryonic stem cells, or gene therapy by the creation of a surrogate beta cell. At the present time, xenotransplantation and stem cell therapy are both fraught with logistic, ethical and legal issues. My laboratory is investigating the use of somatic cell gene therapy as an alternate strategy for reversing diabetes. This strategy is based on the engineering of liver cells to synthesise, store and secrete insulin to glucose and other stimuli, thereby regulating patient blood glucose levels without the need for immunosuppression.

In collaboration with Prof. Tuch at Prince of Wales Hospital we have engineered a liver cell line, Huh7ins that responds in a regulated fashion to a glucose stimulus and corrects diabetes in an animal model. These cells are not destroyed by cytokines of the immune system that precipitate diabetes and this, or a similar cell line could possibly be used clinically following encapsulation and transplantation. An alternative strategy that we are also pursuing is the direct delivery of the insulin gene to the livers of diabetic animals. This procedure has resulted in normalisation of blood glucose levels for 500 days in streptozotocin-diabetic rats, storage of insulin in secretory granules and normal glucose tolerance. These studies give hope that gene therapy may be a treatment for Type I diabetes.

TESTOSTERONE DELIVERY - ROUTES OF ADMINISTRATION

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Since the first clinical use of testosterone in 1937 by frequent IM injection there has been a search for more convenient modes of delivery. Low oral bioavailability has led to almost exclusive use of parenteral preparations and the short half-life of testosterone means that it is best delivered by depot preparations.

Despite being available since the 1940's testosterone implants have been curiously neglected as a treatment modality until recent years. A single subcutaneous insertion of four 200 mg pellets maintains adequate testosterone levels in most men for 5 to 6 months. Side effects are infrequent and usually minor (bruising, bleeding, infection) although extrusion of one or more pellets occurs after about 10% of procedures. The major disadvantage of implants is the need for a minor surgical procedure by a trained operator.

Injectable testosterone esters provide reliable and adequate delivery of testosterone. Testosterone enanthate and combinations of esters approximate steady state levels when given weekly but for convenience are usually given second weekly, resulting in supraphysiological levels in the first few days and low levels towards the end of the second week. The recently availability of injectable testosterone undecanoate, which has been used at 12 weekly intervals, is likely to provide a significant advance in injection therapy.

Unlike synthetic 17-alkylated androgens which are potentially hepatotoxic oral testosterone undecanoate is safe. However multiple daily doses are needed and bioavailability is low and erratic. Transdermal preparations offer the same advantages of oral TU with better bioavailability.

The search for a satisfactory transdermal preparation has been hampered by the need for absorption of mg per day. Non-scrotal patches are large and absorption enhancers lead to a high incidence of skin irritation. Testosterone gel is better tolerated than patches but care needs to be taken to avoid inadvertent transmission to female partners or children. Their short duration of action makes patches and gel particularly suitable for initiation of therapy and in situations where rapid withdrawal of therapy
may be required. (e.g. men with treated prostate carcinoma). However they may not provide adequate testosterone delivery in severe androgen deficiency.

041

GENETIC CAUSES OF REPRODUCTIVE FAILURE IN THE MALE
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Infertility affects 1 in 25 Australian men. Of these, 40% present with primary spermatogenic failure (SgF) manifest as a combination of reduced sperm number, motility or structure/function. SgF is a heterogeneous group of disorders in which genetic causes are increasingly being recognized. A greater understanding of such genetic causes is not only essential in the diagnosis and potential treatment of men bearing the condition, but also in understanding the potential implications for children conceived through artificial reproductive technologies. Further, the identification of effective genetic barriers for male fertility represents a valuable tool for the development of novel male gamete based contraceptives. Chromosomal disorders such as sex chromosome aneuploidies and autosomal translocations are seen in 13.7% of azoospermic and 4.6% of oligospermic men. Y chromosome microdeletions account for 3-5% of men with sperm densities of <5 million/ml, and depending on the region of the Y chromosome deleted present with a spectrum of histopathologies ranging from Sertoli cell only to hypospermatogenesis. More recently work from several groups has suggested that smaller deletions within the same region may or may not critically impair fertility depending upon as yet undefined modifiers on the Y chromosome (the Y haplogroup). Despite the development of many specific gene knockout mouse models with male infertility, the identification of critical single gene lesions in humans has proven to be very difficult. Those that have been identified include the cystic fibrosis transmembrane receptor (CFTR) gene which results in congenital absence of the vas deferens, several genes implicated in primary cilia dyskinesia and a group of genes involved in fibroblast growth factor receptor-1 signaling which are involved in Kallmann’s syndrome. To date, however, only a single gene SCP3 has been unequivocally and mechanistically linked to human male SgF in the absence of somatic pathology.

042

ENDOMETRIAL PROPROTEIN CONVERTASE 6: A CRITICAL REGULATOR FOR EMBRYO IMPLANTATION
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Embryo implantation, during which the free-floating blastocyst attaches to and invades the uterus, is vital for mammalian embryo survival and development beyond the blastocyst stage. In women, implantation failure, resulting in embryonic death, is a major cause of early pregnancy loss and female infertility. Implantation failure also limits successful outcome of assisted reproduction (~70% of embryos transferred fail to implant). A better understanding of the molecular mechanisms of implantation is thus critically important in reproductive medicine. Successful implantation requires not only an implantation-competent blastocyst but also an appropriately prepared conducive endometrium. In a broader sense, endometrial preparation for implantation includes (i) differentiation of the endometrium into a receptive state so that at the expected time of implantation, the embryo will be able to attach and adhere to the luminal epithelium, and (ii) conversion of the endometrium into a competent condition so that appropriate tissue responses in the stroma and vasculature, will occur upon the attachment of the embryo, to allow properly controlled trophoblast invasion. We have established that proprotein convertase 5/6 (PC6), a serine protease of the proprotein convertase (PC) family, is a critical endometrial factor for implantation both in mice and primates. PCs control post-translational activation of a range of proteins with important functions (including HIV envelope proteins), and are regarded as “master switch” molecules and potential therapeutic targets (eg. for combating HIV infection). We propose that PC6 regulates endometrial function by activating a cohort of proteins of diverse functions essential for implantation. This presentation will discuss our current understanding of PC6 function in the endometrium and the potential implications of targeting PC6 for fertility control.

043

ENVIRONMENTAL INFLUENCES ON DNA METHYLATION IN EMBRYONIC CELLS: INVESTIGATING MECHANISMS AND PHENOTYPIC CONSEQUENCES.
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The epigenetic reprogramming in DNA methylation that occurs in the preimplantation embryo appears vulnerable to disruption when in vitro embryo production technologies are applied and may also be influenced by maternal nutrition in vivo. Thus we reasoned that blastocyst-derived, human embryonic stem cells isolated and cultured through a diverse range of protocols in different laboratories (hESC), may also be subject to epigenetic instability and variation (Allegrucci et al., 2004. Lancet 364; 206-20), providing a novel model for the human embryo (Allegrucci et al., 2005. Reproductive Toxicology 20; 353-367). In
order to define the degree of epigenetic variation between independently-derived hESC lines we have employed Restriction Landmark Genome Scanning (RLGS) to examine the genome-wide methylation profiles of gene-rich CpG islands in hESC. Using NotI/EcoRV/HinfI digestion, our comparisons of hESC CpG islands to a normal human lymphocyte profile (comprising 2025 fragments for which a genomic NotI/EcoRV library was available) have revealed significant epigenetic variation between lines that cannot be accounted for by inherent genetic variability. Studies on the effect of a range of culture conditions revealed epigenetic instability over time in culture, with evidence of stable inheritance of changes occurring at lower passage number. The majority of loci which changed over time within a line were not in common between lines, suggesting that passage–associated changes are stochastic and unpredictable. In contrast, common methylation “hotspots” were identified as changing within the BG01 line when deviations from the standard protocol of culture on mouse embryonic fibroblast feeders with passage by manual dissection were applied. We are currently investigating the phenotypic and therapeutic consequences via examining the derivatives of human embryonic stem cells subjected to different environments expected to influence methyl cycle metabolism. Our data thus far suggest that further optimisation and standardization of hESC culture conditions is urgently required to ensure production of biosafe therapeutic products. Since the environmental factors implicated in altering DNA methylation in hESC cultures are in common with a range of human embryo culture media, hESCs might provide a novel model system to optimise culture conditions for assisted reproduction technologies.

In a complimentary approach to examine phenotypic consequences of methylation-relevant nutrients on programming the embryo in vivo, we have developed a sheep model. Maternally-applied methyl group deficiencies in diet during the periconceptual period are being examined for the effects on DNA methylation and subsequent conceptus development. Our rationale for these studies is that identification of key nutrients which can predispose early embryonic cells to programmed epigenetic change might uncover mechanisms pertinent to the developmental origins of adult disease. The latest results of these studies will be presented.

044

A MITOCHONDRIAL COMPONENT TO DEVELOPMENTAL PROGRAMMING

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Adult physiology is not simply dependent on the sequence of the nuclear genes we inherit. There is an increasing appreciation that very early environmental factors can determine development and adult phenotype 1. Recently, in experimental models it has been clearly demonstrated that environmental stress during pregnancy, particularly during the peri-conceptual period is a determinant of adult disease 2. Although these findings substantiate the concept of developmental programming — a process by which very early stress is proposed to have detrimental effects on offspring health. The molecular mechanisms of this phenomenon remain unclear. However, epigenetic changes to nuclear DNA (nDNA) have been implicated in this process 3.

We have evidence that programmed deficits in mitochondrial function contribute to adult disorders in several diverse models of developmental programming. Mitochondria require both nDNA and mitochondrial DNA encoded transcripts to function 4. Therefore persistent changes to either genome could cause abnormal mitochondria. Although we find that different maternal nutrient stresses result in similar deficits in mitochondrial function in adult offspring, namely abnormalities in the activity of mitochondrial electron transport complex iii, we have recently found that this deficit can arise from the environmental disruption of one of a number of distinct cellular processes. Oocytes and preimplantation embryos are particularly prone to environmentally induced changes to mitochondria whose effects can be mimicked in vitro and persist after the initial period of stress 5. The cause and consequences of these changes will be discussed. Our work has particular relevance to assisted conception procedures and to embryo based stem cell derivation.


METHYLCYTOSINE DEAMINATION BY DNA DEAMINASES AND EXPRESSION IN REPROGRAMMING TISSUES.

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Highly differentiated and specialised gametes undergo epigenetic remodeling essential to reconstitute the diploid nucleus of the zygote and restore totipotency. The remodelling involves DNA methylation and covalent modifications of core histones.

DNA methylation is important for epigenetic regulation of the genome. Through normal development it contributes to parental imprinting, X chromosome inactivation, silencing of transposable elements, and tissue-specific gene expression. It is also implicated in cancer and ageing. DNA methylation reprogramming occurs after fertilisation during early mammalian development and during germ-cell development. The loss of methylation without replication seen in these cases suggests an enzyme-catalysed reaction.

The loss of methylation from cytosine may occur directly but is energetically unfavourable. More likely is the deamination of methylcytosine to thymine followed by repair of the mismatch replacing methylcytosine with cytosine. The deamination of methylcytosine can also lead to mutations. With the discovery of cytosine deaminases and the otherwise paucity of methylcytosine directed activities, we are interested in determining whether these deaminases possessed any methylcytosine deaminase activity. We find that Aid and Apobec1 have robust deaminase activities against methylcytosine measured in both an in vitro assay and an E. coli based bioassay. These two deaminases are located in a cluster with other genes expressed in pluripotent tissues, and we find expression of these deaminases in oocytes, primordial germ cells and other pluripotent tissues. We are investigating the role of these deaminases in mice.

DNA demethylation is part of the epigenetic reprogramming that takes place in the early embryo and the establishment of the germ line. Understanding epigenetic events during these key phases of development will contribute to our knowledge of a process that is required for normal development and reproduction, and may prove useful in attempts to treat cancer, defer ageing, and reprogram somatic cells through nuclear transfer.

ORALS - SRB submissions

BONE MORPHOGENETIC PROTEIN-4 IMMUNOLOCALIZATION IS DEVELOPMENTALLY REGULATED IN MICE TESTIS

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The transforming growth factor β (TGF β) superfamily consists of more than 35 proteins including growth differentiation factor (GDF), bone morphogenetic proteins (BMP), TGF-β, activin and inhibin. In males, insitu hybridization studies have shown expression of BMP-2, BMP-4, BMP-8A, BMP-8B in testis and BMP-4, BMP-7, BMP-8A in epididymis of mice (Shimasaki et al., 2004). The aim of our study was to localize BMP-4 protein in testis, epididymis, vas deferens, seminal vesicle and prostate of mice using immunohistochemistry. Tissue samples were collected from male mice aged 1, 2, 4, 7, 9 and 20 weeks (adult). In 1 week old mice, BMP-4 staining was not observed in testis. BMP-4 specific staining was detected in spermatocytes at 2, 4 and 7 weeks but was not detected in testis from 9 week old as well as adult mice. In contrast, BMP-4 specific staining was detected in epithelial cells of epididymis at all stages of testicular development. In the vas deferens, BMP-4 expression was limited to epithelial cells. BMP-4 specific staining was also observed in epithelial lining of the prostate gland but was not detected in seminal vesicles. These findings indicate that BMP-4 play a major role in the initiation phase of murine spermatogenesis, which is further supported by the compromised fertility of BMP-4 heterozygous knockout males (Hu et al., 2004).

(1) Shimasaki S, Moore RK, Otsuka F & Erickson GF 2004 The Bone Morphogenetic Protein System in Mammalian Reproduction. Endocrine Reviews 25 72-101

RELAXIN DEFICIENCY DOES NOT RESULT IN DEVELOPMENTAL ABNORMALITIES IN THE PROSTATE GLAND OF ADULT MICE
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The peptide hormone relaxin prepares the female reproductive tract for parturition, but it may also affect development of the male reproductive tract. Relaxin is produced by the prostate gland and secreted into the seminal plasma in several species. However, its physiological role in the prostate has not been established. This is due, in part, to contradictory results from two recent studies in male mice deficient in relaxin (Rlx<sup>-/-</sup>) or relaxin receptors (Lgr7<sup>-/-</sup>). Rlx<sup>-/-</sup> mice had fewer secretory cells and delayed prostate growth compared to wildtype controls. In contrast, there were no abnormalities in prostate development in Lgr7<sup>-/-</sup> mice. However, neither study examined the different lobes of the prostate. This study therefore re-assessed the phenotype in the prostate of Rlx<sup>-/-</sup> mice at several stages of adult life, differentiating between the anterior, dorsal and lateral lobes. Initial experiments demonstrated gene transcripts for two relaxin-family receptors, LGR7 and GPCR135, in the prostate. However, there was no clear evidence of any relaxin ligand expression. Comparisons of the anterior, dorsal and lateral prostate lobes between Rlx<sup>-/-</sup> and Rlx<sup>+/+</sup> mice at 2, 4, 6, 8 and 12 months of age demonstrated no obvious differences in the duct morphology or epithelium. There was also no apparent difference in the collagen density of the extracellular matrix surrounding the ducts. Adult male Rlx<sup>-/-</sup> mice were chronically infused with human relaxin (3 mg/ml) for 14 days, and the effects on the prostate duct epithelium and stromal extracellular matrix were compared with saline-treated controls. Relaxin treatment did not significantly (P>0.05) affect epithelial cell height in the anterior, dorsal or lateral lobes of the prostate. Furthermore, there was no change in the collagen density of the extracellular matrix. These data are in agreement with the phenotype of the Lgr7<sup>-/-</sup> mouse, and confirm that prostate development in adult mice is not regulated by relaxin.

THE EFFECTS OF OESTROGEN ON MAMMALIAN SEXUAL DETERMINATION
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The interaction of genetic and hormonal pathways is critical for the establishment of sex in mammals. In contrast to the majority of eutherian mammals, sexual differentiation in marsupials such as the tammar wallaby, Macropus eugenii, does not occur until after birth. Birth normally occurs after an active gestation period of 26.5 days, but ‘premature’ births can occur on day 25 of gestation. In males, testis cords form by day 2 post partum (pp) and androgen synthesis begins at about the same time, while in females, the ovaries do not develop cortical and medullary regions until around day 8 pp (Renfree et al. 1992; 1996). Daily oral administration of oestradiol-17β to male pouch young born prematurely after 25 days of gestation induces formation of an ovarian structure in the presumptive testes (Coveney et al., 2001; Renfree et al., 2001). When gonads from male fetuses at day 25 gestation were cultured in DMEM with oestrogen for six days, they developed ovarian-like cortical and medullary–like structures, while male controls developed normal testicular architecture. Real-time PCR analysis of the cultured gonads after oestrogen treatment showed that SRY was dramatically down-regulated as were the downstream male differentiation genes SOX9 and AMH. This suggests that sex reversal is achieved by a loss of SRY signaling in the presence of exogenous oestrogen.

CHARACTERIZATION AND EXPRESSION PATTERNS OF WNT4 DURING GONADAL DEVELOPMENT IN THE MARSUPIAL, MACROPUS EUGENII
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WNT4 is a key regulator of gonadal differentiation in humans and mice and is highly conserved in vertebrates. WNT4 has been proposed as both a female sex-determining and as an anti-testis gene in mammals. Here, we report the characterization of WNT4 from the tammar wallaby (Macropus eugenii), a small Australian marsupial of the kangaroo family. In marsupials, gonadal differentiation occurs post-natally. Before birth, relative WNT4 expression was similar in indifferent male and female gonads. After birth, WNT4 was dramatically increased during ovarian differentiation, peaking by day 9-13 pp when the ovarian cortex and medulla are first distinguishable, then steadily decreased until day 41-49 (when all the germ cells have entered meiosis). In contrast, WNT4 mRNA was down-regulated in testes after birth, as cords started to form, then rose gradually after day 8. This detailed analysis of WNT4 expression in both sexes during the period of gonadal differentiation confirms that WNT4 functions not only as an anti-testis gene during early development, but is also necessary for later ovarian and testicular function in marsupials as it is in mice.
PRESENCE OF TGF-β BUT NOT IL-8 OR GM-CSF IN RAM SEMINAL PLASMA
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The inflammatory response induced in the female reproductive tract following insemination is believed to facilitate maternal immunotolerance to the conceptus. In mice, transforming growth factor-beta (TGF-β) in seminal plasma is largely responsible for this reaction, via the synthesis and secretion of granulocyte-macrophage colony-stimulating factor (GM-CSF) from endometrial epithelial cells. TGF-β has also been identified in seminal plasma of humans and pigs. In mice and humans, 70% - 80% of seminal TGF-β is present in a latent form, whereas in pigs most is present in an active form. This study investigated the presence of TGF-β, GM-CSF and interleukin-8 (IL-8) in ram seminal plasma. Semen was collected by electro-ejaculation from seven rams on three occasions (day 1, day 10 and day 30). Total and active TGF-β1 concentrations were measured using a TGF-β1 ELISA kit (Promega). GM-CSF and IL-8 were measured with a capture ELISA using ovine specific monoclonal antibodies (Serotec). TGF-β1 was found in seminal plasma from all rams on all occasions, but neither GM-CSF nor IL-8 were detected. Mean total TGF-β1 concentration was 400 ± 67 pg/ml (range 120-1500 pg/ml). Of this, 90% was in a latent form. There were significant differences (P<0.05) in total TGF-β1 between different rams, and no active TGF-β1 was detected in the three rams with lowest total TGF-β1 concentrations. TGF-β1 concentrations in ram seminal plasma were much lower than reported in other species. In conclusion, this study found TGF-β1 in ram seminal plasma, but it is uncertain what role seminal TGF-β has in the influx of leukocytes in the reproductive tract of ewes following insemination.


THE SENSE OF SMELL IN THE REPRODUCTION OF THE TAMMAR WALLABY (MACROPUS EUGENII)
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Odours are important to synchronize behaviour and hormones of the potential mating partners in many mammals. In tammar wallabies, odour or pheromones may be important for the acute rise in plasma testosterone concentrations that we have observed in males at the start of the breeding season. They may also be important to assist the neonate in its navigation from the birth canal to the teat. We therefore studied the response of adult males to odours from females and the development of the vomeronasal organ (VNO) from fetus to adult by light microscopy and immunohistochemistry. Females come into oestrus and mate about 1 hour after birth, but are receptive for up to 8 hours. During this time males spend considerable time sniffing the urogenital opening (UGO) and the pouch of females. Male tammars were therefore tested for their preference for swabs taken from the UGO or pouch of oestrous females compared to swabs taken from non-oestrous females. Males (n=8; p=0.488) did not show preference for either oestrus or non-oestrus samples taken from the pouch, whereas males appeared more interested in UGO samples from oestrous females than those from samples of non-oestrous females (n=8; p=0.059). Thus odours from the UGO may be important for the sexual checking behaviour of male tammars. Tammars have a well developed olfactory epithelium and VNO, connected to the nose and mouth via the nasopalatine ducts. The VNO is anatomically well developed before birth. Cells were positive for G-protein coupled receptors in the VNO epithelium at day 15 and 30 of age but not on the day of birth. In contrast, cells in the main olfactory epithelium (MOE) stained positively for olfactory receptors at birth. This makes it likely that the MOE is functional at birth and might assist the neonate in detecting the teat.

THE EFFECT OF POLY-L-LYSINE AND URINE VOLUME UPON THE ADHESION OF NUMBAT AND DIBBLER SPERM TO MICROSCOPE SLIDES.
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Sperm can be found in the urine of a number of Australian marsupial species that show seasonal sperm production, and this has been used in the breeding of captive animals by identifying males that are producing sperm and introducing them with females at the time of oestrus. Whilst sperm in urine can be seen reliably on wet preparations, stained slides are desirable wherever possible as a permanent record of events. The present study has therefore attempted to improve the visualization of sperm from urine of Dibblers (Parantechinus apicalis) and Numbats (Myrmecobius fasciatus) on slides stained with Diff-Quik. In both species, there was a significant increase in the number of sperm seen on slides coated with poly-L-lysine compared with untreated slides (both p<0.005). Interestingly, there were different profiles for both species when looking at the number of sperm seen after the application of increasing volumes of urine. As expected, the number of sperm seen for the Numbat increased with increasing urine volume (p<0.0001). However, this was in contrast to the Dibbler where there was a decrease in sperm seen with increasing urine volume (p<0.0005). The osmolality of the Dibbler urine was in the order of 2735 mOsM, compared to the Numbat urine of...
about 650 mOsM. Dilution of the Dibbler urine with water had a significant effect upon the number of sperm seen after staining despite the same number of sperm being applied to the microscope slide, such that increasing numbers of sperm were seen when the urine was more dilute (p<0.00005).

It is concluded that the adhesion to microscope slides of sperm can be improved (a) for both Numbats and Dibblers by pre-treating the slides with poly-L-lysine, and (b) for Dibblers by keeping to a minimum the effects of the concentrated urine by using small volumes of diluted urine.

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**OXYGEN, INSULIN-LIKE GROWTH FACTOR-II (IGF-II) AND THEIR INTERACTIONS IN MURINE TROPHOBLASTS IN VITRO**

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The low oxygen environment experienced by the early conceptus is essential for placentation. The Hypoxia Inducible Factors (HIFs -1 and -2) mediate the response to low oxygen, inducing genes involved in glucose transport, angiogenesis and cell migration. IGF-II is critical for placental growth and function and can feedback to increase HIF-1 activity in normoxia. We aimed to determine the effects of oxygen and exogenous IGF-II on murine eutocoplacental cone (EPC: trophoblast stem cells) outgrowth and mRNA expression.

EPCs were dissected and cultured for 3 days in 20%, 5% or 1% O₂ with or without the addition of IGF-II and their area of outgrowth quantified. RNA was extracted and the expression of Hif1α, Hif2α, Igf2, Glut1, Vegf, antisense (as) HIF-1α (asHif1α: negative regulator of HIF-1α) mRNA and 18s rRNA was quantified by real-time RT-PCR.

EPC outgrowth was reduced by 35% following culture in 1% compared with 20% O₂. This contrasts with the observation that low O₂ promotes human trophoblast migration. Igf2 mRNA expression was also reduced by culture in 1% O₂. Although the HIFs are generally regulated at the protein level, Hif1α but not Hif2α, mRNA was decreased with decreasing O₂ concentrations. asHif1α mRNA was increased in EPCs cultured at 1% O₂ which may contribute to the decrease in Hif1α mRNA observed after 3 days exposure to low O₂. Nevertheless, Glut1 mRNA was increased with decreasing O₂ concentrations. Exogenous IGF-II had no effect on EPC outgrowth or on Hif1α or Igf2 mRNA expression, although it reduced Hif2α, asHif1α, Glut1 and Vegf mRNA in EPCs cultured at 1%, but not at 20% or 5% O₂. This contrasts with previous studies where IGF-II increased HIF protein expression and HIF mediated transcription. It is likely that the duration of exposure to low O₂ conditions affects HIF mediated responses and has implications for important pregnancy complications.

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**THE EFFECTS OF OXYGEN CONCENTRATION AND GESTATIONAL AGE ON EXTRAVILLOUS TROPHOBLAST OUTGROWTH FROM FIRST TRIMESTER VILLOUS EXPLANTS**

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In the first trimester of human pregnancy, extravillous trophoblasts from anchoring placental villi invade the decidua and transform the maternal spiral arteries. Extravillous trophoblasts temporarily occlude the spiral arteries preventing maternal blood flow and creating a low oxygen environment, which is believed to play an important role in the regulation of extravillous trophoblast outgrowth from villi during the first trimester. Therefore, the purpose of this work was to investigate and quantify the effects of gestational age, oxygen concentration, and the interaction between these factors, on extravillous trophoblast outgrowth from first trimester villi. A quantitative two-dimensional villous explant model was used to measure the frequency and area of extravillous trophoblast outgrowths from 3963 explants grown in 1.5% and 8% oxygen conditions. Gestational age affected outgrowth independently of oxygen concentration, with the percentage of explants producing outgrowth declining as gestational age increased from 8 to 12 weeks. Culture in 1.5% oxygen significantly reduced the frequency and area of extravillous trophoblast outgrowths in comparison to culture in 8% oxygen. The decreased area of extravillous trophoblast outgrowth in 1.5% oxygen can be predominantly attributed to a decrease in the area of individual extravillous trophoblasts, however factors influencing trophoblast proliferation and differentiation may also contribute. Gestation also influenced the response of explants to low oxygen conditions with a significant differential response to oxygen concentration in placenta under 11 weeks of gestation whereas, in villi from placentae of 11 or 12 weeks there was no differential response to oxygen concentration. Thus, in the first trimester oxygen and gestational age both play an important regulatory role in extravillous trophoblast outgrowth.
THE FUNCTION AND EVOLUTIONARY SIGNIFICANCE OF GENOMIC IMPRINTING IN THE MARSUPIAL PLACENTA
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The presence of a fully functional, albeit short lived, placenta in marsupials provides a unique opportunity to assess the importance of genomic imprinting in mammalian placentation. As yet, no studies have addressed the function of any imprinted gene in marsupials. We examined the expression of the imprinted genes IGF2, IGF2R, and P57KIP2, and the imprint status of INS in the yolk sac placenta of the tammar wallaby, Macropus eugenii. IGF2 protein localised to vascular and avascular regions of the chorio-villetine placenta, but it was more highly expressed in the vascular region. IGF2 expression initially increased and then declined in the late gestational stage placenta, but this profile was not mirrored by IGF2R. In eutherians P57KIP2 is co-expressed with IGF2, but is down regulated by increased IGF2 protein levels. In the tammar wallaby, P57KIP2 immunostaining and mRNA expression paralleled IGF2. The addition of IGF2 to cultured placental explants did not significantly alter P57KIP2 expression in the tammar. These differences may account for the divergent imprint status of P57KIP2 in marsupials and eutherians. Insulin expression is paternally biased in the placenta, but not in the embryo. INS protein is located in the vascular and avascular regions, with strong staining in placent al endoderm in late gestation. In the tammar wallaby there is a marked increase in prostaglandin production by the placenta immediately before birth and insulin may be crucial for this placental function. Since these genes appear to have a similar function in these widely separated mammalian groups, our results suggest a common origin for genomic imprinting in marsupial and eutherian mammals.

PROTEOMIC ANALYSIS OF HUMAN PLACENTA IDENTIFIED INCREASED EXPRESSION OF CHLORIDE INTRACELLULAR CHANNEL 3 WITH PRE-ECLAMPSIA
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The aim of the study was to use 2D PAGE and mass spectrometry to compare the proteomes of human placental samples from pre-eclampsia (PE) with control pregnancies, and to use immunoassay to confirm differential expression of selected proteins. The fetal circulation and corresponding intervillous space of single cotyledons of placentas from women with PE (either with or without fetal growth restriction, FGR) or from control women were perfused with a modified Krebs solution. Maternal effluent samples were subjected to 2D PAGE (1st D: pH3-10, 17cm; 2nd D: 10-20% gradient) and LC-Ms/Ms (ESI-trap) was performed on protein spots that showed significant differential expression (PDQuest v7, Biorad; Mann Whitney, 95% CI) between PE and controls. Eight proteins that were increased and two that were decreased with PE were matched to known sequences (Mascot search engine). One of the proteins with increased expression significantly matched to Chloride Intracellular Channel 3 (CLIC3) a member of the CLIC family of proteins which regulate the intracellular movement of chloride. Western blot analysis showed a single band at 26kDa for placental extracts, as well as for recombinant CLIC3 protein. ELISA measured significantly increased (ANOVA, p<0.001) concentration of CLIC3 in placental extracts from pregnancies with PE and FGR (922±509ng/mg total protein, n=6) compared to both gestation-matched controls (204±71, n=17) and PE without FGR (188±48, n=22). CLIC3 proteins are involved in a number of fundamental cellular processes including the stimulation of apoptotic processes in response to cellular stress. CLIC3 has previously been shown to facilitate chloride uptake in transfected fibroblast cells. The results demonstrate that 2D gel-based proteomic analysis of human placenta can identify differentially expressed proteins with a pregnancy disorder and that placental expression of CLIC3 is increased with the combined pathologies of PE and FGR. Altered CLIC3 expression may play a role in abnormal placental function with PE.

CASPASE-14: A NEW PLAYER IN CYTOTROPHOBLAST DIFFERENTIATION
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Caspase-14 is the most recently discovered member of the caspase family of proteins; however the role for caspase-14 in apoptosis remains uncertain. It has however been shown to be involved in keratinocyte differentiation and cornification. We believe that caspase-14 has a conserved role in cellular differentiation as a modified form of apoptosis and therefore propose a role for caspase-14 in differentiation and fusion of the cytotrophoblast. The human choriocarcinoma BeWo cell line was treated with Staurosporine and Forskolin to induce apoptosis and differentiation respectively. Apoptosis was confirmed by 3'-end labelling and the JC-1 assay, while differentiation was determined by Real Time Quantitative PCR for βhCG and E-cadherin mRNA. Staurosporine initiated apoptosis within 3 hours of treatment, while late stage apoptosis occurred around 6 hours after treatment. Beta-hCG mRNA expression was significantly increased at all stages following Forskolin treatment...
indicating biochemical differentiation, while E-cadherin mRNA was decreased 72 hours after treatment indicating morphological differentiation. Caspase-14 mRNA was found to be unchanged in apoptotic BeWo cells, but elevated in differentiating BeWo cells at all time points. Therefore caspase-14 is involved in biochemical differentiation of the cytotrophoblastic BeWo cell line. Moreover, caspase-14 may interact with other signalling molecules and facilitate the process of differentiation. We have previously reported the presence of caspase-14 in the human trophoblast and its role independent of apoptosis. This new data confirms the potential for the BeWo cell line in the functional dissection of this unusual caspase and its prospective role in trophoblast differentiation.

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CYCLIC AMP STIMULATES SOLUBLE VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR 1 (sVEGFR-1) PRODUCTION IN HUMAN CYTOTROPHOBLAST.

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Soluble vascular endothelial growth factor 1 (sVEGFR-1), which is a potent biological antagonist for both vascular endothelial growth factor (VEGF) and placental growth factor (PIGF), is expressed during placentation development. We previously showed that the level of serum sVEGFR-1 was elevated in women with preeclampsia1. However, the regulatory mechanism of sVEGFR-1 expression in trophoblast is poorly understood. The aim of this study was to investigate whether cyclic AMP (cAMP), which is known to stimulate cytotrophoblast differentiation, affected sVEGFR-1 production by human cytotrophoblast.

Placental tissues (5-8w) were obtained from women who underwent induced abortion. Cytotrophoblasts were isolated by trypsin and DNase digestion, and purified by density gradient centrifugation. Cytotrophoblasts were cultured for up to 96 h in the presence or absence of dibutyryl cAMP (0.1 mM, 1 mM), forskolin (1 µM, 10 µM) or protein kinase A inhibitor H-89 (5 µM), in both normoxic (20 % O2) and hypoxic (2 % O2) conditions. Cytotrophoblast production of sVEGFR-1 and human chorionic gonadotropin (hCG) was determined by measuring culture media with enzyme-linked immunosorbent assay (ELISA) and enzyme immunoassay (EIA), respectively.

Either cAMP or hypoxia significantly increased sVEGFR-1 production by cytotrophoblast in a dose- and a time-dependent manner. H-89 partially inhibited the cAMP induced-sVEGFR-1 production. On the contrary, cAMP upregulated, whereas hypoxia downregulated, the production of hCG.

Although cAMP and hypoxia have opposite effects on hCG production, both stimulated sVEGFR-1 production in human cytotrophoblast. These results suggest that production of sVEGFR-1 in human cytotrophoblast is regulated by cAMP and oxygen tension, independently of their effects on the trophoblast differentiation.

(1) Koga et al. (2003) JCEM. 88, 2348-51

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OPPOSING EFFECTS OF HGF AND TGF-B ON HLX1 EXPRESSION IN HUMAN TROPHOBLAST CELLS

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Trophoblast proliferation is critical for normal placentation development. Abnormal trophoblast proliferation is a characteristic feature of various pregnancy disorders such as fetal growth restriction and pre-eclampsia. The homeobox gene HLX1 is a transcription factor that controls proliferation in embryonic cell types. It is well established that HLX1 is expressed in trophoblast cells but its role in the placenta is not well understood. Several growth factors and cytokines have been proven to play a pivotal role in regulating trophoblast function. We have previously shown that HLX1 regulates the important trophoblast cell function of proliferation and that colony stimulating factor-1 (CSF-1) acts through HLX1 to control trophoblast proliferation (submitted). In this study we hypothesized that hepatocyte growth factor (HGF) and transforming growth factor (TGF-β1) are also important for HLX1 regulating trophoblast functions. The well characterised human trophoblast cell line SGHPL-4 was used in this study. Following HGF stimulation, HLX1 mRNA expression was significantly increased in cultured trophoblast cells (p<0.001, n=3) whereas TGF-β1 stimulation resulted in a significant decrease in HLX1 mRNA expression (p=0.001, n=3). Trophoblast proliferation, as measured by tritiated thymidine uptake, significantly increased with HGF stimulation (p<0.001, n=6), however, upon stimulation with TGF-β1, trophoblast proliferation was significantly reduced (p<0.001, n=6). Therefore, HGF and TGF-β1 have opposing effects on HLX1 mRNA expression levels and on trophoblast proliferation.
THE EFFECT OF MATERNAL PROTEIN DURING PREGNANCY ON BIRTH WEIGHT IN THE BOVINE

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It is clear from a world wide series of epidemiological studies in human populations and experimental studies in a range of animal models that varying maternal nutrition during critical periods of fetal development can alter or ‘program’ body mass and body composition in later life (1-3). There has been a major experimental focus on determining the impact of low protein diets on experimental laboratory animals but few on large ruminants of agricultural importance. Protein is the most deficient nutrient in the Australian Rangelands. To determine if low dietary protein concentration in the first two trimesters of pregnancy alters calf growth and development, heifers were inseminated with semen from the same bull on a single day and allotted to four treatment groups. These were fed high or low protein diets during the first and second trimesters (Low/Low n=19, Low/High n=17, High/Low n=18, High/High n= 17).

Preliminary results show that there was no effect of nutrition in trimester 1 on calf birth weight (p=0.524) but there was a significant effect of nutrition in trimester 2 (p=0.017) High nutrition in the second trimester resulted in heavier calves (means ± se of 33.0 ± 0.68 and 30.8 ± 0.62 for high and low respectively). Further, results on the effects of these nutritional effects on placental development, milk production and quality, ADG, carcass quality, will soon be available.

High birth weight is of commercial importance as it is associated with dystocia in the heifer. Feto-pelvic disproportion accounts for the majority of losses in extensively managed herds including nine (13%) heifers in this trial. As well as economic loss for the grazer of the calf and or heifer there are welfare considerations which need to be addressed in the effective management of the heifer herd.

RESTRICTING UTERINE BLOOD FLOW IN LATE PREGNANT RATS RESULTS IN AN INCREASE IN UTERINE RELAXIN RECEPTOR (LGR7) EXPRESSION

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Two important functions of the peptide hormone relaxin in pregnant animals are uterine growth and proliferation of blood vessels in the endometrium. The G-protein coupled receptor Lgr7 binds relaxin with high affinity, and is expressed in the myometrium and cervix of mice, and endometrial epithelium in humans. A similar receptor, Lgr8, is also expressed in reproductive tissues but the relative amounts of both receptors have not been assessed in the same tissue. In addition, no studies have identified physiological factors that regulate uterine Lgr7 expression. The first aim of this study was to differentiate between Lgr7 and Lgr8 gene expression in the reproductive tract of pregnant rats. We then tested the hypothesis that restricting uterine blood flow will alter uterine Lgr7 gene expression in late pregnant rats. This was achieved by bilateral ligation of the maternal uterine blood vessels in Wistar Kyoto rats on day 18 of their 22-day gestation (n=7). A second group of animals was sham operated as controls (n=6). Tissues were collected two days later on day 20 gestation. RT-PCR and quantitative PCR established that Lgr7 gene expression was significantly higher in the uterus, placenta and cervix compared with Lgr8 in late pregnant rats (P<0.01). Uteroplacental restriction (UPR) resulted in a significant decrease in fetal weight and litter size (P<0.05). It also caused a significant increase in uterine Lgr7 gene expression compared with controls (P<0.05). Regression analysis between litter size and uterine Lgr7 mRNA concentrations showed that in control animals, larger litter sizes were correlated with lower uterine Lgr7. In contrast, increased litter size in the UPR animals was correlated with increased uterine Lgr7 expression. These data show that in UPR rats, uterine Lgr7 gene expression may be up-regulated to compensate for the reduction in uterine blood flow in late pregnancy.

SYSTEMIC MATERNAL AWARENESS OF CONCEPTUS ANTIGENS IN PREGNANCY

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The conceptus expresses paternal antigens including classical and non-classical MHC but does not experience immunological attack. Emerging evidence suggests rejection is avoided by active maternal immune tolerance towards conceptus antigens. However, the mechanisms responsible for establishing tolerance, including if and how conceptus antigen primes the maternal immune system, remain undefined. We have developed T-cell transgenic models using ovalbumin (OVA) as a model paternal antigen to investigate the kinetics and location of processing and presentation of paternal antigens in priming the maternal immune system during pregnancy. Transgenic Act-mOVA male mice expressing OVA driven by the β-actin promoter were mated to C57BL/6 females, to generate conceptuses expressing OVA in OVA-deficient females. Pregnant mice received CFSE-labelled OVA-reactive OT-1 T cells on either days 1, 4, 7, 11 or 15 of pregnancy. T cell proliferation and CD69 expression in OT-1 cells were quantified as a gauge of the extent of OVA antigen processing and presentation 3 days after transfer. OT-1 cells given on day 1 of pregnancy showed marked levels of activation and proliferation, in a response limited to the para-aortic lymph nodes draining the uterus but not seen in the spleen or other peripheral nodes. OT-1 cells received on day 4 of pregnancy displayed very low levels of activation and proliferation in all lymphoid tissues, including the para-aortic lymph nodes. Over days 7, 11 and 15 of pregnancy an increasing number of OT-1 cells became activated as proliferation progressively intensified, indicating high levels of OVA antigen processing and presentation. Antigen presentation occurred earliest and most intensively in the para-aortic nodes, and over the course of pregnancy progressively spread to peripheral sites including the mesenteric and cervical lymph nodes and the spleen. The data show that paternal-derived antigens associated with both semen and the conceptus actively prime the female immune system, with the response becoming strong and systemic through mid and late gestation. This provides a mechanism whereby semen exposure provides the initial priming event for paternal antigen recognition in pregnancy, and the placenta sustains this response after implantation. Exploiting the model will allow investigation of the antigen recognition events underpinning establishment and maintenance of the maternal immune tolerance facilitating pregnancy success.
INVESTIGATION OF MATERNAL IMMUNE FUNCTION THROUGHOUT PREGNANCY
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Suppression of Th1 immune responses is important for successful pregnancy. The transcription factor Nuclear Factor Kappa B (NF-κB) is a key regulator of Th1 immune responses. We have previously shown that the level of NF-κB protein is downregulated in CD3+ T cells isolated from third trimester pregnant women relative to those isolated from non-pregnant women. The aim of this project was to investigate the expression of NF-κB p65 and p50 mRNA and protein in CD3+ T cells throughout pregnancy. T cells were isolated from non-pregnant, first, second and third trimester pregnant women (n=10 each group) and subjected to RNA extraction using Trizol™ and whole cell lysate protein extraction. NF-κB p65/p50 mRNA and protein expression was assessed by semi-quantitative RT-PCR and Western blot respectively. Our results showed that the level of p65 gene expression was similar in non-pregnant and first trimester pregnant women. In contrast, the level of p65 gene expression was reduced in second and third trimester pregnant women relative to non-pregnant women. This was confirmed by analysis of p65 protein expression in T cells from pregnant and non-pregnant women. The level of p65 protein was similar in non-pregnant and first trimester samples and was reduced in second trimester pregnant women compared to non-pregnant women although not significantly (p=0.06). The level of p65 protein was significantly reduced in third trimester pregnant women relative to non-pregnant women (p<0.05). In contrast, the level of p50 mRNA was similar in all groups analysed. Our data suggests that the suppression of NF-κB p65 mRNA and protein occurs in the second trimester of pregnancy and continues into the third trimester.

MATERNAL INSULIN-LIKE GROWTH FACTOR-I AND -II ACT VIA DIFFERENT PATHWAYS TO INCREASE FETAL GROWTH NEAR TERM
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The placenta transports substrates and wastes between the maternal and fetal circulations. Perturbed placentation can lead to pregnancy complications for which there are no preventative treatments. In mice, placental insulin-like growth factor (IGF)-II is essential for normal placental development and function, but in other species, maternal circulating IGF-II is substantial and may also contribute. Maternal circulating IGFs increase in early pregnancy and correlate with placental functional development and fetal growth in women and guinea pigs. This study determined the effect of maternal IGF-I and IGF-II supplementation in early pregnancy on fetal growth, placental development and maternal body composition near term in the guinea pig. Pregnant guinea pigs were infused with IGF-I, IGF-II (both 1mg/kg/day) or vehicle subcutaneously from days 20 to 38 of pregnancy (n=7-9 per treatment) and killed on day 62 (term=69 days). IGF-II, but not IGF-I, increased the mid-sagittal area and volume of placenta devoted to exchange by ~30%, the total volume of trophoblast and maternal blood spaces within the placental exchange region (+29% and +46%, respectively) and the total surface area of placenta for exchange by 39%. Both IGFs reduced resorptions and IGF-II increased the number of viable fetuses by 26% and increased fetal weight by 11-17%. IGF-I, but not IGF-II, reduced maternal adipose depot weights by ~30%. In conclusion, increased maternal IGF-II abundance in early pregnancy promotes fetal growth and viability near term by increasing placental structural and functional capacity, while IGF-I appears to divert nutrients from the mother to the conceptus. This suggests major and complementary roles in placental and fetal growth of the increased circulating IGFs in early to mid pregnancy.

MATERNAL IGF TREATMENT IN EARLY TO MID PREGNANCY HAS SUSTAINED EFFECTS ON PLACENTAL TRANSPORT & NUTRIENT PARTITIONING NEAR TERM
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Appropriate partitioning of nutrients between the mother and conceptus is a major determinant of pregnancy success, with placental transfer playing a key role. We have previously shown in the guinea pig that increased maternal insulin-like growth factor (IGF) abundance in early-mid pregnancy increases fetal development and viability near term (1). IGF-II, but not IGF-I, enhanced placental structural differentiation and increased the proportion of the placenta devoted to exchange near term. Here we show the effect of increased maternal IGF in early pregnancy on placental transport and nutrient partitioning in midgestation (day 35) and near-term (day 65, term=69 days). Pregnant guinea pigs were infused with IGF-I, IGF-II (both 1mg/kg/day) or vehicle subcutaneously from day 20 to 38 of pregnancy and tissue uptake and placental transfer of the non-metabolisable radioanalogues [3H]-methyl-D-glucose (MG) and [14C]-amino-isobutyric acid (AIB) and plasma metabolite concentrations in mother and fetus measured. IGF-I, but not IGF-II, increased placental and fetal weight (+13% and +11%) and MG and AIB uptake by the placenta (+42% and +68%) and fetus (+59% and +90%) in mid-gestation. Both IGFs increased fetal plasma and tissue MG (+40-50%) and IGF-I increased placental MG uptake (+70%) near term. Both IGFs increased fetal plasma amino acid concentrations (+130%) and IGF-I reduced fetal plasma cholesterol (-30%) near term. In the mother, IGF-I increased MG and AIB uptake by muscle in mid- and late-gestation and both IGFs increased MG and AIB uptake by visceral
organs near term. In conclusion, increased maternal IGF exposure during early to mid pregnancy persistently increases placental delivery of nutrients to the fetus for growth throughout pregnancy. This occurs despite increased maternal utilisation and may involve enhanced placental function.


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**HTRA3, A SERINE PROTEASE, IN HUMAN PREGNANCY SERUM.**

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HtrA3 is a member of the HtrA family of serine proteases (HtrA1-4). We have previously demonstrated that HtrA3 is closely associated with placental development both in the mouse and human (1, 2, 3). In women, HtrA3 protein is located primarily in the glands and decidual cells throughout the cycle but its expression peaks during the implantation window. Furthermore, glandular and decidual cell HtrA3 expression is elevated further during pregnancy. HtrA3 is also present in specific trophoblast subtypes in the first trimester placenta. In this study we determined whether: 1) HtrA3 is detected in the maternal serum during pregnancy, 2) HtrA3 levels in sera change during pregnancy and 3) whether the profile is different in sera collected from women diagnosed with pregnancy-related disorders compared with gestation controls.

To investigate HtrA3 levels in pregnancy sera, we have optimised western blots using an in house anti-HtrA3 antibody to detect HtrA3. On comparison with endometrial tissue derived protein, serum HtrA3 appears smaller according to SDS-PAGE, suggesting further modification of the protein. Similar to endometrial HtrA3, serum HtrA3 levels rise dramatically with pregnancy compared with non-pregnant sera. Furthermore, the HtrA3 profile changes during pregnancy with the appearance of an additional 30 kDa band in second trimester sera samples, followed by diminished protein levels in the third trimester. HtrA1 was also detected in pregnancy sera but did not mirror the HtrA3 profile. We are currently using the above methods to screen sera to determine whether HtrA3 is a useful marker for pregnancy-related disorders. These findings demonstrate that serum HtrA3 levels parallel growth and development of the placenta and may be a useful indicator of placental health.

1 Nie et al., 2006 Biol Reprod 74:366-374
2 Nie et al., 2005 Placenta 27:491-501
3 Nie et al., 2003 Mol Hum Reprod 9: 279-290

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**CHARACTERIZING THE RELAXIN RECEPTOR (RXFP1) AND THE RELAXIN SIGNALING PATHWAY IN HUMAN UTERINE CELLS.**

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The heterodimeric peptide hormone relaxin acts through a G-protein-coupled receptor called RXFP1 (previously LGR7). This receptor has a large extracellular domain comprising 10 leucine-rich repeats, as well as an LDL-binding motif and a cysteine-rich “cap” structure. The full-length extracellular domain (ectodomain) is capable on its own of binding relaxin with high affinity, giving significance to the expression of RXFP1 splice variants, some of which encode this domain in a soluble form. We have generated antibodies against two regions of this ectodomain, as well as against the third intracellular loop of this receptor, and applied these in an immunohistochemical study of the human and primate uterus, to determine the localization of expressed receptor protein. Using all three antibodies provided convincing controls validating the specificity of the findings. Staining was observed particularly on stromal cells, close to endometrial glands, in the early secretory phase. Weak staining was also observed on some epithelial cells as well as in the myometrium. RT-PCR analysis of isolated myometrial, stromal and epithelial cells, indicated the presence in all three cell types of RXFP1 gene transcripts, predominantly as the full-length transcript.

Primary human endometrial cell cultures have been established to explore the role of relaxin in endometrial differentiation. In particular, the signalling components activated upon relaxin stimulation of endometrial stromal cells have been intensively studied. These results are compared with RXFP1-mediated signal transduction in the relaxin-responsive THP-1 monocyte cell-line. 1) Compared to transfected cells, over-expressing RXFP1, natural receptors appear to use additional cell-specific amplification systems; 2) naturally expressed receptors require additional cytoplasmic components involving an essential tyrosine kinase activity in order to achieve up-regulation of cAMP production; and 3) this results in a sustained production of intracellular cAMP, necessary for the persistent endometrial differentiation essential for implantation and pregnancy.
INCREASED EXPRESSION OF SUPPRESSORS OF CYTOKINE SIGNALLING (SOCS) IN THE RAT OVARY DURING PREGNANCY
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Prolactin (PRL) and Placental Lactogen (PL), both acting via the PRL-receptor, play essential luteotrophic roles in the rodent corpus luteum (CL) of pregnancy. Towards the end of pregnancy, luteolysis is induced by PGF2α, which reverses many of the effects of PRL-receptor signalling on gene expression in the CL. Our earlier research on Day 19 pregnant rats has shown that an early event in luteolysis is the inhibition of PRL-receptor signalling, most likely caused by upregulation ofSuppressors of Cytokine Signalling (SOCS) proteins (1). In the present study, we examined endogenous SOCS expression in the rat ovary during pregnancy.

Mature female Wistar rats were mated (vaginal plug = Day 1 pregnancy) and ovaries were collected on every third day of pregnancy from Day 7 to Day 19 (N=5 per day). After euthanasia, ovarian tissue was removed and frozen, before RNA extraction (TRIzol), DNase treatment, reverse-transcription (Superscript), and amplification by quantitative Taqman real-time PCR. SOCS mRNA expression in each sample was normalised relative to beta-Actin mRNA expression.

The mRNA expression of all SOCS examined (SOCS1, SOCS2, SOCS3, and CIS) significantly increased from early (Day 7) to mid-pregnancy (Day 10 to 13). After mid-pregnancy SOCS expression remained significantly elevated through to Day 19, except SOCS1 which was highly variable between animals. Notably, a significant increase in mRNA expression for SOCS3 and CIS was observed between Days 16 and 19.

In summary, SOCS mRNA expression in the rat ovary increases markedly at mid-pregnancy and either remains at relatively high levels (SOCS2), or further increases (SOCS3 and CIS) in later pregnancy. Increased SOCS expression is most likely due to PL secretion that begins around Day 10 and is sustained through pregnancy. Whether the additional increase in SOCS3 and CIS mRNA expression in late pregnancy is due to increasing levels of PGF2α remains to be proven.

(1) Curlewis et al. 2002 Endocrinology 143:3984-3993.

EXPRESSION OF FOLLISTATIN LIKE -3 IN DEVELOPING MOUSE GONADS
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Members of the transforming growth factor-b (TGFb) superfamily influence many aspects of reproduction. Their influence is modulated by a variety of proteins that act both outside the cell to reduce ligand binding, and inside the cell to limit signal transduction. Follistatin-like 3 (Fstl3) is an extracellular glycoprotein that can reduce signalling by binding to activins and to bone morphogenetic proteins. Fstl3 is produced in adult gonads, but nothing is known about Fstl3 in embryonic gonads. Since TGFb superfamily signals influence many aspects of fetal gonadogenesis, we hypothesized that knowledge of Fstl3 expression would reveal sites where regulation of activin and BMP signalling occur. Here we describe the expression of Fstl3 in Swiss mouse fetal gonads. RT/PCR demonstrated expression of Fstl3 in both male and female gonads from embryonic day 14.5 (E14.5)-E18.5. Cellular expression of the Fstl3 mRNA was detected with a digoxigenin-labelled cRNA probe corresponding to a domain unique to Fstl3, using a combination of whole mount and section in situ hybridization. Male gonads from E12.5 to birth and through to adulthood were examined. Fstl3 expression was widespread and consistently observed in mitotic germ cells and in several somatic cell types, with distinct signals also noted in elongating spermatids. Immunohistochemistry on Bouin’s-fixed, paraffin-embedded sections and on paraformaldehyde-fixed frozen sections showed Fstl3 protein in gonocytes of the developing testis and in the Sertoli cell cytoplasm. Notably, at E14.5, protein was detected in the gonocyte cytoplasm, but nuclear staining was evident at E16.5 and at the day of birth. At this period, the gonocytes are mitotically arrested. These data indicate that Fstl3 contributes to regulation of TGFb superfamily signalling at all stages of testis development, and its differential intracellular localisation may be linked with changes in germ cell activity.

THE LETHAL PHENOTYPE IN RELAXIN-DEFICIENT (RLX-/-) MICE IS DUE TO ABNORMAL NIPPLE GROWTH, AND NOT IMPAIRED MAMMARY GLAND STRUCTURE OR FUNCTION.
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The peptide hormone relaxin is essential for nipple development during late pregnancy in rodents. In relaxin-deficient mice (RLX-/_), the nipples are abnormally small, therefore pups are unable to receive milk and die within 24 hours of birth. Relaxin is also thought to stimulate mammary gland growth, but its functional importance in milk production has not been examined. This study measured relaxin and relaxin receptor (Lgr7) gene expression in the mouse ovary and mammary gland at different stages of pregnancy and early lactation. We then tested the hypothesis that relaxin is involved in milk protein production as well as mammary gland growth in mice. RT-PCR demonstrated that relaxin was expressed in the ovary during late gestation but not lactation. However, there were no relaxin or Lgr7 gene transcripts expressed in mammary tissues. Comparison between
Rlx+/+ and Rlx/- mice showed no obvious differences in either lobuloalveolar structure or ductal branching in the mammary gland. However the nipple was markedly smaller in Rlx/- mice. Mammary explants from Rlx/- mice expressed β-casein in response to lactogenic hormones at a similar level to Rlx+/+ mice, implying normal milk protein production. Pregnant mice chronically infused for 3 days with human relaxin (0.1 mg/ml/h) from day 15.5 gestation gave birth to live pups without difficulty on the morning of day 19. The pups were able to suckle at the mothers' nipples immediately after birth, with pups surviving beyond 24 hours. The nipples of the relaxin-treated mice were significantly more developed than saline-infused controls, but there were no growth effects of relaxin on mammary tissue. In summary, mammary gland structure is not altered in Rlx/- mice and relaxin is not critical for normal milk protein synthesis. The lethal phenotype in Rlx/- mice therefore results from insufficient milk delivery caused by inadequate nipple development.

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**NULL MUTATION IN TRANSFORMING GROWTH FACTOR BETA1 IMPAIRS MAMMARY GLAND DEVELOPMENT AND IMPEDES LACTATION**

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Transforming growth factor beta 1 (TGFbeta1) is a multifunctional cytokine involved in many aspects of biological function, particularly in regulation of the immune system, and during development. The mammary gland is a unique organ in that almost all of its development occurs postnatally, during puberty as the rudimentary epithelial duct extends and branches to form a large ductal network, and during pregnancy as the ability to produce and secrete milk is acquired. We have studied the effect of a null mutation in the TGFbeta1 gene on mammary gland development in mice. TGFbeta1 deficiency causes severely impaired mammary gland development. Mammary ductal branching was found to be considerably reduced, with fewer branch points evident in six of six virgin 12 week old TGFbeta1 null females compared to TGFbeta1 replete females, and in two of six the gland had not extended to the edge of the fat pad. Two TGFbeta1 null females that gave birth to live TGFbeta1 replete pups were unable to feed the pups. The pups failed to thrive and died. A third litter were surrogated to a normal lactating female and survived to adulthood. These results suggest TGFbeta1 is a critical factor in promoting mammary gland development, during both puberty and pregnancy, and are contrary to previous findings using TGFbeta1 transgenic models, that find that TGFbeta1 is a growth inhibitory cytokine in mammary gland development.

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**INSULIN FAMILY RECEPTORS IN THE DEVELOPING MARSUPIAL**

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Sex determination in mammals is dependent upon the presence of the sex determining *SRY* (sex-determining region on the Y) gene on the Y-chromosome. However, the complex sexual differentiation pathway in mammals is enigmatic and is still not fully characterised. In addition to *SRY*, other genes are progressively being discovered that play a role in sexual differentiation. A recent study in mice of a triple knockout of three insulin family receptors suggests a critical role for these genes in mouse sex determination and the regulation of *SRY*. In the tammar, the gonads of both sexes are indeterminate at birth and testis cords form between day 1 and day 2 postpartum (pp). In our study, we partially cloned insulin receptor (*IR*), insulin-like growth factor-1 receptor (*IGF-1R*) and insulin receptor-related receptor (*IRR*) sequences from the tammar. The receptor proteins were widely distributed in the gonads and were expressed in both male and female fetuses and neonates from at least one day before birth to one day after. Expression of these receptors precedes the peak in *SRY* expression during the critical period when testis differentiation is occurring. This suggests that IR, IGF-1R and IRR may play a crucial role in male sexual differentiation similar to the mouse, and that they could be involved in *SRY* regulation. Insulin family receptors have not yet been identified in any mammal other than the mouse, so the high degree of conservation in sequence and expression profile in this marsupial suggest that these genes are critical for normal sexual differentiation.
DISRUPTION OF HEDGEHOG SIGNALLING IN THE ADULT MOUSE TESTIS
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Hedgehog (Hh) signalling is known to regulate many aspects of normal development as well as being upregulated in various cancers. Although deletion of one Hedgehog ligand, Dhh, leads to male infertility, the role that Hedgehog (Hh) signalling plays in the adult testis has not been addressed. We previously identified the expression patterns of Hh signalling pathway components in the adult mouse testis by in situ hybridisation, and confirmed the presence of the Desert Hh (Dhh) ligand, receptors, various cytoplasmic regulators and the Gli transcriptional mediators. To examine the role of this signalling pathway in adult spermatogenesis, we have developed a novel adult mouse testis tubule culture method in which to test the impact of reduced Hh signalling on cell proliferation, viability and gene expression. In this system, cyclopamine was administered to inhibit Hh signalling in testis tubule segments of defined length cultured in hanging drops for 48 hours. Cyclopamine is a natural alkaloid derivative of the plant Veratum californicum which inhibits Hh signalling by targeting the Smoothened receptor. Expression of Gli1, a known Hh target gene, is strongly downregulated, confirming inhibition of the signalling pathway as previously reported for cyclopamine treatment. To search for other downstream Hh target genes, RealTime PCR measurements were used to compare mRNA expression levels of candidate genes between control and cyclopamine treated cultures. Preliminary results have identified igfbp6 and cyclin D2 as genes regulated by Dhh. The discovery of downstream target genes regulated by Dhh signalling will provide vital clues to the function of this pathway in the postnatal testis.

REGULATION OF SPERMATOGONIAL PROLIFERATION BY INTERLEUKIN-1 AND ACTIVIN A IN VITRO: A RE-EXAMINATION USING AN ANTAGONIST APPROACH
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Interleukin-1α (IL-1α) and activin A are produced within the seminiferous epithelium in a regulated cyclical manner and control spermatogonial proliferation and differentiation. Since it remains unclear precisely how these cytokines interact, we investigated the effects of recombinant human IL-1α and activin A, and their respective antagonists, IL-1 receptor antagonist (IL-1ra) and follistatin (FS), on [3H]-thymidine incorporation into staged adult rat seminiferous tubule fragments (2 mm) in short-term cultures (16h, 32°C). Cells displaying DNA synthesis were identified by immunohistochemistry in parallel paraffin-embedded bromodeoxyuridine (BrdU)-labeled tubule fragments. Across the cycle, peak [3H]-thymidine incorporation was observed at stages IV-V and late stage VII-XI, with barely detectable incorporation at early-mid stage VII. Addition of IL-1α (20-40 U/ml) or activin A (10-50 ng/ml) did not affect incorporation within these stages. IL-1α (1 µg/ml) inhibited incorporation in fragments of stage IV-V (intermediate and type B spermatogonia), while FS (100 ng/ml) stimulated late stage VII and VIII (preleptotene spermatocytes). These data suggest that endogenous IL-1α stimulates proliferation of B spermatogonia, while endogenous activin A inhibits preleptotene spermatocytes. These data also present a different picture to that provided by several earlier studies: both IL-1α and activin A stimulated DNA synthesis by preleptotene spermatocytes in longer term (48h) tubule fragment cultures1, or in Sertoli-spermatogonial cell co-cultures4. Other studies, however, have shown that activin inhibits and FS stimulates FSH-induced spermatogonial proliferation in testis fragment cultures from younger rats2,4. Altogether, the data indicate opposing effects of endogenous IL-1α and activin A on spermatogonial development at different stages of the cycle, and highlight the complexity of this regulation, which can produce different experimental outcomes under different experimental conditions.

(1) Meehan et al. 2000 Developmental Biology 220: 225
(2) Boitani et al. 1995 Endocrinology 136: 5438
(3) Mather et al. 1990 Endocrinology 127: 3206
(4) Hakovirta et al. 1993 Endocrinology 133: 1664

RAM INTRODUCTION STIMULATES PULSATILE LH SECRETION IN CYCLIC EWES
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Introduction of rams to anoestrous ewes stimulates an almost instantaneous increase in LH pulse frequency and this usually leads to an induced ovulation, a phenomenon termed the ‘ram effect’ (review: Martin et al., 1986)1. Application of the ram effect during the breeding season has been previously disregarded because the reproductive endocrine axis is powerfully inhibited by progesterone during the luteal phase (Walkden-Brown et al., 1999)2. However, anoestrous ewes under the influence of exogenous prostagens respond to ram introduction with an increase in LH concentrations (Evans et al., 2004)3.
suggesting that cyclic ewes at different stages of a naturally occurring luteal phase would also show an increase in LH pulse frequency following ram introduction.

During January (early breeding season), the oestrous cycles of adult Merino ewes (n = 24) were synchronised using intravaginal progestagen pessaries. Pessary insertion and withdrawal were staggered to produce three groups of ewes (n = 8) at different stages of the luteal phase; early luteal, mid-luteal and late luteal, with each group balanced for age (6-7 years) and weight (55-57 kg). On the day of ram introduction, blood was sampled every 12 minutes for 3 hours before and 2 hours after ram introduction.

Ewes at all stages of the luteal phase showed a significant increase in LH pulse frequency in response to ram introduction (Table 1). Furthermore, ewes in the early and mid-luteal phases showed an increase in basal LH concentrations. There was no effect of ram introduction on LH pulse amplitude (Table 1).

In conclusion, ram introduction appears to override or bypass the steroid feedback mechanism that normally maintains a low LH pulse frequency during the luteal phase in cyclic ewes. We hypothesise that this change in the endocrine milieu may impact on follicle dynamics and oestrous cycle length.

Table 1. Characteristics of the LH profiles of ewes before and after ram introduction at different stages of the luteal phase.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Early luteal</th>
<th>Mid luteal</th>
<th>Late luteal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulses per hour</td>
<td>0.21 ± 0.06</td>
<td>0.29 ± 0.08</td>
<td>0.17 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>0.85 ± 0.13***</td>
<td>1.19 ± 0.09**</td>
<td>1.00 ± 0.15***</td>
</tr>
<tr>
<td>Pulse amplitude (ng/ml)</td>
<td>0.51 ± 0.06</td>
<td>0.38 ± 0.08</td>
<td>0.34 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>0.54 ± 0.10</td>
<td>0.55 ± 0.10</td>
<td>0.20 ± 0.03</td>
</tr>
<tr>
<td>Basal concentration (ng/ml)</td>
<td>0.20 ± 0.09</td>
<td>0.21 ± 0.04</td>
<td>0.21 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>0.37 ± 0.04*</td>
<td>0.35 ± 0.07*</td>
<td>0.23 ± 0.02</td>
</tr>
</tbody>
</table>


DEVELOPMENT OF ANTERIOR PITUITARY CELLS AND COLOCALISATION OF TSHβ AND FSHβ WITH LHB-IMMUNOREACTIVITY IN THE LATE GESTATIONAL SHEEP FETUS AFTER DISCONNECTION OF THE HYPOTHALAMUS AND PITUITARY

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Despite our knowledge of hypothalamic regulation of the anterior pituitary (AP) after development, the role of the hypothalamus in influencing the developmental profile of AP cells remains largely unknown. In order to evaluate total hypothalamic contributions to AP cell development, we utilised the technique of hypothalamo-pituitary disconnection (HPD) in sheep fetuses. HPD or sham surgery was performed at 110 days gestation (d) (n=6 each group; Term 147±3d). Fetuses were delivered and pituitaries collected at 110d (no surgery group) or 141d (sham and HPD groups). The objective was to assess the impact of HPD on AP cell development by single-labeled immunofluorescence for each of the six AP hormones. In addition, we determined the colocalisation of the glycoprotein TSHβ in gonadotrophs using double-labeled immunofluorescence. We found that HPD was associated with a 70% increase (P<0.05) in relative thyrotroph numbers compared to sham. In contrast, HPD prevented the gestational age-related increase in gonadotrophs (+147% compared to 110d; P<0.05). The gestational age-related increase in lactotrophs (+80%) was not affected by HPD. The relative proportions of somatotrophs and corticotrophs were unaffected by either gestational age or HPD. Because HPD caused opposite changes in thyrotroph and gonadotroph cell populations, we investigated the effects of HPD on a population of TSH/gonadotropin-expressing 'stem' cells. Dual-labeled LHβ/TSHβ cells were extremely rare (<1%) and the changes in numbers of cells expressing TSHβ or LHβ occurred independently of the presence of other hormones. Together, these results indicate that AP cells are capable of developing in the absence of an intact hypothalamo-pituitary connection after 110d, but that hypothalamic factors are essential for maintenance of appropriate proportions of thyrotrophs and gonadotrophs. Additionally, colocalisation for LHβ and TSHβ is rare and unaffected by HPD in late gestation, suggesting that any hypothalamic influence on cellular expression of glycoprotein hormones occurs independently in thyrotrophs and gonadotrophs.
DIFFERENTIATING THE SITES OF ACTION OF TESTICULAR STEROIDS IN THE REGULATION OF GnRH SECRETION AND MATING BEHAVIOUR IN RAMS: A MODEL
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In males, testosterone acts in the brain to regulate reproduction, through inhibition of gonadotrophin-releasing hormone (GnRH) secretion, and through stimulation of mating behaviour. In both cases, the main site of action is within the brain, but the precise location is largely unknown. The aim of this study was to develop a model through which to differentiate the site(s) of these two actions of testosterone in the brain of the ram. Much of the action of testosterone is through the metabolites, 5α-dihydrotestosterone (DHT) and/or oestradiol 17β. Adult Merino wethers (n=5/group), castrated as lambs, were injected (im) twice daily for 32 days with peanut oil, 8mg DHT benzoate (DHTB), 25μg oestradiol benzoate (EB) or both DHTB and EB. Jugular blood samples collected every 15 minutes for 10h revealed that all 3 treatments significantly (P<0.001) suppressed plasma LH concentrations (EB 0.80±0.35, DHTB 0.17±0.15, EB+DHTB 0.38±0.23, Oil 1.55±0.33 ng/ml ±SEM). Mating tests with oestrous ewes repeated 4 times over 9 days revealed that wethers treated with oil or DHTB showed little or no mating behaviour, while EB treated wethers consistently exhibited courtship-type behaviour (genital sniffing, tongue flick, nudge/foreleg kick and flehmen, but generally without mounting). By contrast, all the wethers treated with both EB and DHTB exhibited all the above behaviours but with frequent mounting, intromission and ejaculation. Thus, while both DHT and EB were able to suppress LH secretion, DHT alone had no effect on mating behaviour, EB elicited courtship behaviour only, while combined EB and DHT elicited a full sexual response. From this study, we have a model that can be used to examine the brain areas involved in the actions of testosterone in the brain of males.

(withdrawn).

MODULATION OF ADRENAL RESPONSIVENESS IN GESTATING FEMALES OF EGERNIA WHITII, A VIVIPAROUS SKINK
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In reptiles and mammals, exposure to elevated corticosteroids during embryonic development may have significant effects on offspring fitness. In many mammals, gestating females down-regulate their acute response to stressors to protect current reproductive investment. This study aimed to determine if adrenocortical function is similarly modulated during gestation in the viviparous lizard, Egerinia whitii. We compared the adrenocortical response to acute capture stress in female E. whitii during early (post-ovulatory) and late gestation, and during the post-partum phase of the reproductive cycle. In viviparous reptiles, oestrogen is highest during late vitellogenesis, and drops markedly during early gestation. To explore the potential for oestrogen as a modulator of HPA axis function, we compared the responses of post-partum females, oestrogen-treated females and reproductively inactive males to an ACTH challenge.

In E. whitii, females' responses to acute capture stress varied significantly with reproductive stage. Post-partum females displayed the most marked response, with a rapid and sustained increase in plasma corticosterone (CORT) concentrations, while post-ovulatory females showed the most conservative adrenocortical response to acute capture stress. During late gestation, females showed a marked immediate response, but this was attenuated compared with that of post-partum females. Reproductively quiescent males and postpartum females exhibited similar responses to acute capture stress, and responded similarly to ACTH injection, with plasma CORT reaching maximal concentrations of 52.1 ng/mL and 59.4 ng/mL respectively. Postpartum females treated with oestrogen exhibited greater responsiveness to ACTH than non-treated females, although basal plasma CORT concentrations were unaltered: these results suggest that the attenuation of the acute stress response observed in gestating females of E. whitii may be regulated upstream of ACTH secretion. Our results demonstrate that in this viviparous reptile the activity of the HPA axis is modulated by reproductive state, and provides evidence that gestating females may buffer their embryos from the potentially adverse effects of elevated plasma corticosteroids.
THE CONTRACEPTIVE MECHANISM OF LEVONORGESTREL IN A MARSUPIAL SPECIES
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Subcutaneous hormone implants are a useful method for management of overabundant marsupials. Levonorgestrel (a gestagen) induces long-term infertility in the tammar wallaby and koala. In humans the contraceptive actions of levonorgestrel are well characterized, yet in marsupials, the mechanism by which levonorgestrel prevents pregnancy is unknown. The mechanism of action in kangaroos and wallabies is complicated by embryonic diapause, since pregnancy resumes after the conceptus has been arrested for varying at the blastocyst stage. In this study, ovarian development of female tammar wallabies was monitored after insertion of a single levonorgestrel or control implant at the start of pregnancy. Twenty levonorgestrel treated and 16 control animals were autopsied the day before birth (day 25) and the accompanying post-partum oestrus, while 10 levonorgestrel treated and 9 control animals were autopsied 3-4 days (days 29 – 30) after the expected birth and oestrus. Levonorgestrel treatment did not prevent follicular growth, as there was no significant difference in follicle size at day 25 between treatment and control animals. Levonorgestrel treatment blocked ovulation as none of the treated females autopsied at days 29 – 30 had ovulated (n=10), in contrast to 80% (4/5) of the controls. Levonorgestrel inhibited mating in most, but not all, treated females. However, 30% (3/10) did mate, as evidenced by the presence of copulatory plugs This study suggests that levonorgestrel suppresses the release of gonadotrophins, and blocks ovulation and oestrus in this species, as it does in eutherian mammals.

AN EFFECTIVE SUPEROVULATION PROTOCOL FOR THE MARMOSET MONKEY (CALLITHRIX JACCHUS)
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²Research Centre for Reproductive Health, University of Adelaide, Woodville, SA, Australia
³Department of Reproductive Biology, German Primate Centre, Goettingen, Germany
⁴Reproned Adelaide, Dulwich, SA, Australia

The ability to collect large numbers of developmentally competent marmoset oocytes in a single stimulated cycle will greatly facilitate embryo study in this species. The aim of this study was to characterize the ovarian response to FSH-priming with or without hCG administration. Cloprostenol (0.8 µg), a prostaglandin F₂α analogue, was administered between days 11 and 18 of the luteal phase. Recombinant human FSH (Gonal-F) was injected twice daily (25 IU/injection) for the next 6 days and 500 IU hCG (Pregnyl) was injected at 14:00 h on the day after the final FSH injection. Ovaries were removed at 10:00 h on the day after FSH-priming (FSH alone; 7 animals) or the hCG injection (FSH + hCG; 4 animals). Cumulus-oocyte complexes (COCs) were recovered from small (SA; 0.7-1.5 mm) and large antral (LA; >1.5 mm) follicles. COCs with expanded cumulus cells were parthenogenetically activated or inseminated to assess their developmental potential. Total COCs recovered per animal from SA (28.3 ± 7.7 to 26.8 ± 1.9) and LA (22.6 ± 6.3 to 33.3 ± 13.8) follicles did not differ significantly between the stimulation groups. Stimulation with FSH + hCG increased the number of expanded COCs recovered from LA follicles per animal (23.5 ± 9.3 v 6.4 ± 2.7) and the proportion of LA-derived COCs that had undergone cumulus expansion (71% v 29%) compared with FSH alone. Nearly all the oocytes in expanded COCs were mature at the time of isolation (117/125; 94%). Together, these data indicate that the hCG administration induced in vivo maturation of oocytes in LA follicles. Following insemination or parthenogenetic activation, cleaved embryos formed blastocysts at rates of 26% (7/27) and 0% (0/25), respectively. This study demonstrates that the FSH + hCG protocol described induces an effective ovarian response in marmosets that yields large numbers of developmentally competent, in vivo matured oocytes.

PROSTAGLANDIN E₂ UP-REGULATES LUTEINIZING HORMONE RECEPTOR (LHR) EXPRESSION AND ENHANCES STEROIDOGENIC RESPONSES OF FOLLICLE CELLS
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Influence of the male on female reproductive behaviour and physiological processes are widespread among mammals. This study examines the mechanisms underlying the novel observation that factor(s) in semen advance ovulation in pigs (Waberski et al., J Reprod Fertil 1997, 109:29-34 ). Our recent observations that uterine exposure to seminal plasma increased endometrial cyclo-oxygenase-2 (COX-2) expression (O'Leary et al., Reproduction 2005, 128: 237-247) and augmented steroidogenic response of isolated pig follicle cells to luteinizing hormone (LH) (O'Leary et al., Reproduction 2006, in press)
led us to investigate actions of prostaglandin E$_2$ (PGE$_2$), a major product of COX-2, on LH receptor (LHR) activity and steroidogenic responses in isolated follicle cells. Granulosa cells isolated from follicles >5mm in diameter were cultured in vitro with PGE$_2$ and hCG alone and in combination. Cyclic AMP secretion in response to PGE$_2$ and hCG was determined as a measure of functional LHR expression. Receptor-coupled physiological responses to these agonists were assessed by measurement of progesterone secretion. Both PGE$_2$ and hCG significantly increased cAMP secretion by granulosa cells, indicating expression of adenylate cyclase-linked receptors for both agonists. Culture with PGE$_2$ increased LHR expression by 5-fold in granulosa cells (100 ± 18 fmol/1000 cells) compared to controls cultured without PGE$_2$ (20 ± 8). Culture with PGE$_2+$hCG resulted in a 5-fold increase in progesterone secretion compared to hCG alone (392 ± 8 pmol/1000 cells vs. to 92 ± 12), providing evidence that the increased LHR was coupled to a steroidogenic functional response in granulosa cells. In conclusion, PGE$_2$ increases LHR expression and coupled steroidogenic response in granulosa cells of pig follicles. The findings support the hypothesis that PGE$_2$ serves as an intercellular mediator by which signals generated in the uterus as a result of semen exposure in pigs enhance gonadotrophic hormone actions on follicle cells and lead to advanced ovulation.

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**INHIBIN α SUBUNIT WITH AN A257T MUTATION IS ASSOCIATED WITH PREMATURE OVARIAN FAILURE: IS THIS INHIBIN FORM BIOACTIVE?**

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A naturally occurring missense mutation (769G>A) resulting in a non-conservative change of alanine to threonine in the inhibin a subunit (INHA, A257T) has been shown to be associated with POF. The aim of this study was to demonstrate that this mutation impairs inhibin bioactivity in several *in vitro* systems. Two transfection reporter systems were used to assess inhibin antagonism of activin A-stimulated FSHβ and GnRHR promoter activity in pituitary gonadotroph (LβT2) and ovarian (COV434) cells. Overexpression of the mutant inhibin showed significantly less suppression of FSHβ and GnRHR transcriptional activity in LβT2 cells compared to wild type ($P=0.02$, $P=0.0001$ resp, $n=6$). In COV434 cells, the mutant inhibin was less bioactive than wild type in suppressing GnRHR promoter activity ($P<0.0001$, $n=6$). This decrease in bioactivity was not attributable to reduced inhibin α-β subunit dimerisation based on similar inhibin levels measured by immunoassay in wild type and mutant inhibin cultures. These findings were confirmed by assessing the *in vitro* activity of purified wild type and mutant inhibin A and B forms to suppress FSH secretion in rat pituitary cells. When expressed as a ratio of *in vitro* bioactivity to inhibin immunoactivity, the bioactivity of mutant inhibin B was also reduced. It is concluded that the mutated inhibin α subunit, in dimeric α-β subunit form, is less active than wild type forms in these *in vitro* systems and provides a basis for the higher incidence of POF in women with this mutation. This reduction in inhibin bioactivity could result in an accelerated loss of follicles leading to POF. The current study highlights the importance of inhibin in the female reproductive system and provides further insights into the genetic aetiology of POF.

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**NEUTROPHIL DEPLETION RETARDS ENDOMETRIAL REPAIR**

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Inflammatory cells are highly abundant within the endometrium prior to and during menstruation. We hypothesise that these contribute functionally to menstruation and/or endometrial repair. As menstruation only occurs in women and few other species, lack of suitable animal models makes functional studies difficult. We developed a unique mouse model of endometrial breakdown and repair, in which decidualisation is artificially induced, and progesterone support withdrawn; endometrial tissue progressively breaks down by 24h after progesterone withdrawal and by 48h has usually undergone complete repair, morphologically resembling human endometrium at menstruation. In this study, the presence and localisation of markers for key inflammatory cells were examined in our model, and the functional contribution of neutrophils determined. Immunohistochemistry revealed neutrophils as the most abundant leukocyte. They were rare in decidual tissue, elevated during breakdown and most abundant during early repair. To assess their functional contribution to these processes, the antibody RB6-8C5 was administered to deplete neutrophils. Significant depletion was confirmed both within the circulation (control: neutrophils 22.3±2.4% of total leukocytes, RB6-8C5 treated: 3.8±0.3%, $P=0.0001$) and tissue. To ascertain whether depletion affected breakdown or repair, a morphological scoring system was established; sections were assigned a score between 1 (intact decidual tissue) and 5 (complete repair) based upon key morphological features. For each animal 2-4 cross-sections from different areas of the uterus were scored blind by two independent observers. Scoring of control (n=9 per time point) and neutrophil depleted (n>15) uteri revealed that neutrophil depletion caused some retardation of
endometrial breakdown. However, most notable was the significant (p<0.001) delay in endometrial repair. At 48h, 59% of sections from neutrophil depleted animals were delayed compared to 11% from control animals. These findings demonstrate for the first time using an in vivo model, that neutrophils play an important functional role in the processes of endometrial breakdown and repair.

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HYDROXysteroid sulfOCONJUGATION AS A PUTATIVE DETERMINANT OF FOLLICULAR LUTEINIZATION

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The process of follicular luteinization is characterized by a marked reduction in estrogen biosynthesis. In the present study, it was hypothesized that luteinization also involves the gonadotropin-dependent induction of a gene responsible for the metabolism of the estrogen precursor dehydroepiandrosterone (DHEA). DHEA sulfotransferase (SULT2A1) is responsible for the sulfocojugation of hydroxysteroids, thereby changing their physical properties and preventing their conversion to active estrogens. This enzyme requires the presence of a sulfonate donor molecule called 3'-phosphoadenosine-5'-phosphosulfate (PAPS), synthesized by the enzyme PAPS synthase (PAPSS).

To investigate the regulation of SULT2A1 and PAPSS during follicular luteinization, the equine preovulatory follicle was used as a model. The regulation of SULT2A1 and PAPSS mRNA was studied in preovulatory follicles isolated during estrus at 0, 12, 24, 30, 33, 36 and 39 h (n = 4-6 follicles/time point) after an ovulatory dose of human chorionic gonadotropin (hCG), and in corpora lutea (n = 3) obtained on day 8 of the estrous cycle. Results from RT-PCR/Southern blot analyses showed significant changes in steady-state levels of both SULT2A1 and PAPSS mRNA after hCG treatment (P <= 0.05). Levels of SULT2A1 were low in follicle wall samples prior to hCG treatment and markedly increased in samples obtained at 36 h post-hCG. When analyses were performed on isolated cell preparations, a marked and significant increase in SULT2A1 mRNA was observed 33-39 h post-hCG in granulosa cells (P <= 0.05) and 30-39 h post-hCG in theca interna (P <= 0.05). Levels of PAPSS mRNA did not significantly change in intact follicle wall samples. Nonetheless, when analyses were performed on isolated cell preparations, a significant increase in PAPSS transcript was observed at 39 h post-hCG in granulosa cells (P <= 0.05), with a transient increase occurring at 12 h post-hCG in theca interna (P <= 0.05). Collectively, these results demonstrate the gonadotropin-dependent induction of SULT2A1 and PAPSS mRNA in preovulatory follicles after hCG treatment and suggest that the process of follicular luteinization involves not only the downregulation of genes responsible for estrogen biosynthesis, but also the upregulation of genes involved in the metabolism of an estrogen precursor.

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NOVEL LEUKEMIA INHIBITORY FACTOR ANTAGONIST BLOCKS BlastOCYST IMPLANTATION IN THE MOUSE

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Blastocyst implantation is a critical stage in the establishment of pregnancy. Endometrial leukemia inhibitory factor (LIF) is essential for implantation in the mouse (1), with expression peaking on day 3 (d3) of pregnancy (d0 = plug detection) in the uterine glandular epithelium (2). We tested the effect of a novel LIF antagonist on implantation in the mouse. The potent LIF antagonist MH35-BD (3) was conjugated to polyethylene glycol (PEG) to increase its half-life in vivo. The bioactivity of PEG-MH35-BD was tested using a Ba/F3 cell proliferation assay. Its in vivo half-life in mice was determined by giving a single intraperitoneal (IP) injection (1 mg/kg) of either PEG-MH35-BD or the control PEGylation reagent (mPEG2-NHS), and assaying serum at time points up to 5 days using a mouse LIF ELISA (n = 2/group). PEG-MH35-BD was present in serum from 10 mins to 5 days after injection. There was no detectable LIF in serum from control or untreated mice at any time point. Mated mice (n = 5-6/group) were injected IP with 250 μg (12.5 mg/kg) of either PEG-MH35-BD or mPEG2-NHS on d2 (12 midday and 10pm) and d3 (10am), and the uterus examined for implantation sites on d6. Only mice with visible corpora lutea at d6 were included in the study (n = 4-5/group). No implantation sites were observed in the uteri of PEG-MH35-BD-treated mice, while the control mice had normal numbers of implantation sites (0 (PEG-MH35-BD) vs. 8.8 ± 0.5 (mPEG2-NHS), mean ± SEM). Implantation was not blocked when PEG-MH35-BD was injected IP at a lower dose (6.25 mg/kg) at the same time points, or when the same doses were given 24 hours earlier on d1 and d2. These data demonstrate that a novel PEGylated LIF antagonist completely blocks blastocyst implantation in mice, providing valuable information for the development of new contraceptives for women.


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LASER CAPTURE MICRODISSECTION AND ARRAY ANALYSIS OF ENDOMETRIUM IDENTIFY CCL16 AND CCL21 AS EPITHELIAL - DERIVED INFLAMMATORY MEDIATORS ASSOCIATED WITH ENDOMETRIOSIS


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Endometriosis is an inflammatory condition. Therefore chemokine secretion in endometriosis may offer a novel area of therapeutic intervention. We aimed to identify chemokines differentially expressed in epithelial glands in eutopic endometrium from normal women and those with endometriosis and to establish the expression profiles of key chemokines in endometriotic lesions. Laser capture microdissected epithelial glands from endometrial eutopic tissue from women with and without endometriosis, in the mid-secretory phase of the menstrual cycle, were profiled using a human chemokine and receptor cDNA array. Verification of selected chemokine gene expression used real-time PCR and immunohistochemistry was also performed on ectopic endometriotic lesions. 22 chemokine and receptor genes were markedly upregulated while two were downregulated in endometrial epithelium of women with endometriosis compared with controls. Verification studies complemented this observation and statistical significance was achieved in some instances. Real-time PCR analysis of CCL16 and CCL21 demonstrated increased endometrial CCL16 but not CCL21 expression levels in women with endometriosis compared to controls (P=0.049). Immunostaining for CCL16, not CCL21, was more intense in glands in endometriosis eutopic tissue compared to controls (P=0.001). Furthermore increased CCL21 protein expression was apparent in ectopic lesions compared with matched eutopic tissue in the endometriosis patients (P=0.002). This study provides novel candidate molecules and suggests a potential local role for CCL16 and CCL21 as contributing factors towards endometriosis-related pain and infertility.

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HORMONAL REGULATION AND CONVERTASE ACTIVITIES OF COMPLEMENT 3 IN HUMAN OVUDCTAL EPITHELIAL CELLS

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Human oviduct cells produce complement 3 (C3). The derivative of C3, iC3b, but not C3 enhanced mouse preimplantation embryo development. We hypothesized that the human oviduct uses the complement pathways for complement activation to convert C3 to iC3b via C3b and that production of C3 in the oviducts is under hormonal regulation. The aim of this study is to investigate the effect of hormones on C3 mRNA expression and the conversion of C3 into C3b/iC3b in the human oviductal epithelial cells (OE).

In vitro cultured primary OE cells were treated with various concentrations of estrogen and progesterone either alone or in combination. Estrogen enhanced C3 mRNA expression of OE cells. Progesterone alone has no effect on C3 expression. The presence of the components of C3 convertases were studied by RT-PCR and immunocytochemistry, respectively. Molecules involved in complement activation, C2 and C4 in the classical pathway and lectin pathway, and factor B (fB) and factor D (fD) in the alternative pathway was detected in the OE cells. The OE cell culture possessed active C3 convertase that converted exogenous C3 into C3b in a time-dependent manner. No iC3b was produced under this condition.

In conclusion, the production of C3 in oviduct is estrogen-regulated and the oviductal cells can convert C3 to C3b but not iC3b. Acknowledgements: This work is supported by Research Grant Council, Hong Kong (HKU7319/ 01M , HKU 7411/ 04M ).

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THE ROLE OF IMATINIB IN THE REGULATION OF GRANULOSA CELL TUMOUR CELL GROWTH

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Granulosa cell tumours (GCTs) of the ovary are rare, hormonally active neoplasms characterised by endocrine manifestations, an indolent course, and late relapse. Chemotherapy and hormonal therapy have proved to be of limited efficacy. Highly potent, selective inhibitors of tyrosine kinases are being developed as alternatives to standard chemotherapy. Imatinib mesylate (Gleevec) is routinely used to treat chronic myelogenous leukaemia, and has recently been used successfully to treat a patient with GCT. To evaluate whether imatinib might have a role in the treatment of GCT, we sought to: 1) determine the effect of imatinib on two GCT-derived cell lines; and 2) characterise the pattern of gene expression for targets of imatinib and to screen for known activating mutations. Treatment of both cell lines with increasing concentrations of imatinib reduced both COV434 and KGN cell proliferation and viability. In KGN cells, this was due to a dose-dependent, imatinib-induced apoptosis.

References:

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COV434 cells appear to undergo necrosis. Gene expression patterns of c-ABL, c-KIT, PDGFR-α and -β were determined in a panel of GCT (n=15) and normal ovary (n=10). Uniform expression of c-ABL, c-KIT, PDGFR-α and variable expression of PDGFR-β was observed in normal ovary. Variable expression of c-ABL and both PDGFR was observed in GCT; c-KIT expression was lower than that seen in normal ovary. All genes were expressed in KGN cells; in contrast, only c-ABL was expressed in COV434 cells. Known activating mutations in c-KIT and PDGFR-β were not observed. Our data shows that imatinib can arrest proliferation in GCT-derived cells; the mechanisms involved, however, appear to differ between the two cell lines. Given that high doses of the drug caused this effect, it is likely that imatinib is exerting “off-target” effects. Although imatinib is unlikely to be effective, a tyrosine kinase inhibitor of differing specificity may be able to treat this disease.

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**NOVEL VARIANTS IN HUMAN GDF9 IN MOTHERS OF DIZYGOTIC TWINS**

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Genetic factors contribute to an increased chance of having dizygotic (DZ) twins. Genes from the ovarian bone morphogenetic signalling pathway (GDF9 and BMP15) are critical for normal human fertility. We previously identified a deletion mutation in GDF9 in sisters with spontaneous DZ twins, but the prevalence of rare GDF9 variants in twinning families is unknown. We therefore screened for rare variants in GDF9 in families with a history of DZ twinning. The GDF9 gene was screened in 279 unrelated mothers of DZ twins by denaturing high performance liquid chromatography (DHPLC). Variants were confirmed by DNA sequencing and selected variants typed by MALDI-TOF mass spectrometry in 3376 individuals from 923 DZ twinning families (2317 mothers of twins) and in 1512 controls of Caucasian origin. We found two novel insertion/deletions (c.392-393insT, c.1268-1269delAA) and four missense alterations in the GDF9 sequence in mothers of twins. Two of the missense variants (c.307C>T, p.Pro103Ser and c.362C>T, p.Thr121Leu) were located in the pro-region of GDF9 and two (c.1121C>T, c.3762C>T, p.Thr121Leu) were located in the mature protein region. For each variant, the frequencies were higher in cases compared to controls, with significant differences for c.1268-1269delAA, p.Pro103Ser and p.Pro374Leu. The frequency of all GDF9 variants was significantly higher (P<0.0001) in mothers of twins (4.12%) compared with controls (2.29%). We conclude that rare variants altering the GDF9 protein sequence are significantly more common in mothers of DZ twins than controls suggesting that GDF9 variants contribute to the likelihood of dizygotic twinning.

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**IMPORTIN A2-RECOGNISED NUCLEAR IMPORT IN THE CONTROL OF SPERMATOGENESIS**

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Spermatogenesis is a unique and ordered process that is orchestrated by the precise expression of a specific set of genes at each stage. Progression through successive stages requires the shuttling of proteins and transcription factors into and out of the nucleus to implement changes in gene transcription. Major factors that mediate nucleocytoplasmic transport are members of the importin superfamily, of which there are with five and 20 different mouse importin α and β genes, respectively. Several of these importins display distinct mRNA and protein expression patterns in adult mouse testis indicating that individual importins can carry a specific cargo(es) at discrete stages of spermatogenesis. Identification of these candidate cargoes should describe potential developmental switches that are critical to the spermatogenic process. In a yeast two hybrid screen using an adult mouse testis library and full length importin α2 as bait, we have identified coilin, a component of coiled/Cajal bodies, and Chr8, a cysteine and histidine rich protein, as likely binding partners for importin α2. Both proteins are highly expressed in the testis; and preliminary verification of their interaction with importin α2 has been demonstrated in yeast, co-immunoprecipitation, co-transfection and immunohistochemistry experiments. Coiled bodies are small, round nuclear inclusions that can associate with histone genes. It has been suggested that since coilin can bind to U7 small nuclear ribonucleoprotein, it may facilitate histone pre-mRNA processing by recruiting RNA processing factors to the coiled bodies. Chr8 has been demonstrated to bind to galectin-3, which has also been implicated in pre-mRNA splicing. Therefore, it is likely that the regulated expression and timely importin α2-mediated nuclear transport of these proteins may be important to co-ordinate events that are pivotal in regulating gene expression during spermatogenesis. Continued studies will focus on elucidating the biological significance of these interactions during spermatogenesis.
THE IMPORTIN-A2-DEPENDENT NUCLEAR IMPORT MECHANISM OF PSMC3IP AND ITS POSSIBLE ROLE DURING SPERMATOGENESIS

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Differentiation of spermatogonial stem cells into mature spermatozoa requires changes at the level of the nucleus in gene expression, which may partially be controlled through the regulation of nuclear transport factors, including importins (IMPs). We previously showed that all of the IMPαs (1, 2, 3, 4 and 6) and several IMPβs are developmentally regulated during mouse spermatogenesis, consistent with the idea that individual IMPs have specific regulatory roles in spermatogenesis by transporting particular cargoes at discrete stages of differentiation. To identify key IMPα cargoes we performed a yeast-two-hybrid screen using IMPα2 as bait to interrogate an adult mouse testis cDNA library. Proteasome 26S subunit ATPase 3, interacting protein (PSMC3IP) was identified as a binding partner of IMPα2. PSMC3IP has been reported by others to have significant roles during homologous chromosome pairing and recombination and in modulating 26S proteasomal activity. Using constructs prepared using Gateway™ vectors, IMPα2-PSMC3IP interactions were confirmed in preliminary experiments in transformed yeast and transfected mammalian cells. Importantly, IMPα2 was able to enhance the nuclear accumulation two-fold of PSMC3IP in cotransfected Vero cells, as determined using quantitative CLSM analysis. Furthermore, immunohistochemical analyses of Bouin’s fixed paraffin-embedded sections of adult testis indicated that PSMC3IP is present within spermatocytes and in round and elongating spermatids. Our current hypothesis is that PSMC3IP is transported into the nucleus by IMPα2 during the meiotic stages of spermatogenesis. Elucidating the mechanisms by which proteins such as PSMC3IP access the nucleus and how important this is to the developmental process of spermatogenesis has strong relevance to models of male infertility.

COEXPRESSION AND POTENTIAL INTERACTION OF THE NUCLEAR TRANSPORTER IMPORTIN B3 AND NUCLEAR PORE COMPLEX COMPONENT NUCLEOPORIN NUP153 IN MOUSE TESTIS.

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Spermatogenesis involves multiple cellular transitions to form mature sperm from germ line stem cells, steps which require changes in transcription factors (TF), chromatin remodelling factor and cell cycle regulator action within the nucleus. Nuclear access is generally mediated by importin (IMP) superfamily members, of which there are 5 IMPα and >20 IMPβ identified in mouse. The IMPs recognise specific cargoes and facilitate their passage through the nuclear pore complex (NPC), which is made up of 40-50 nucleoporin (Nup) proteins. We hypothesized that IMP/TF interactions during spermatogenesis represent potential switches in spermatogenic development. This direct interaction may provide an additional regulatory mechanism in sperm differentiation. To find proteins that interact with IMPβ3 during spermatogenesis, we performed a yeast two-hybrid screen of an adult mouse testis library using a truncated IMPβ3 construct as bait. Amongst the positive clones isolated, three independent clones were found to encode portions of the nucleoporin Nup153. This Nup localises to the nucleoplasmic side of the NPC and functions as a mobile Nup, able to move dynamically on and off of the NPC, possibly in response to binding cargo proteins. Immunohistochemical staining of Bouin’s fixed paraffin-embedded testis sections from adult mice, as well as those at days (D) 0, 5, 15, and 20 postpartum (pp), using polyclonal sheep anti-Nup153 antibody (Immunoquest) revealed Nup153 to be present in the germ cells at D0, and in elongating spermatids in the adult, indicating that Nup153 is developmentally regulated. A varying Nup repertoire within the NPC may give altered transport properties, important in modulating spermatogenesis and the ferrying of cargoes. Importantly, immunohistochemical staining also showed IMPβ3 is coexpressed in elongating spermatids, consistent with the concept that IMPβ3 and Nup153 work together to ferry specific cargos into the nucleus in haploid male germ cells.

DIFFERENTIAL EXPRESSION OF ACTIVIN RECEPTORS IN NORMAL, HORMONE-TREATED, AND NEOPLASTIC HUMAN TESTIS.

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Although activin protein is produced locally and influences both germ cell and Sertoli cell function, the cells that make activin receptors are unknown in the human testis. Immunohistochemistry was utilised to localise activin type I (ALK2 and ALK4) and type II (ActRIIA and ActRIIB) receptor proteins in Bouin's fixed, paraffin embedded sections of adult human testis. ActRIIA immunoreactivity was low to undetectable, while ALK2 ALK4 and ActRIIB staining were readily detected in spermatogonia and Sertoli cells. A small subset of spermatogonia exhibited variously nuclear, cytoplasmic, and cell surface expression of ALK2, ALK4 and ActRIIB. We also examined samples showing impaired spermatogenesis, including those from patients with carcinoma in situ (CIS; precursors to testis cancer), seminoma and non-seminoma, and from normal men subjected to hormonal suppression with androgen-based contraceptives. A strong ALK2 signal was evident in CIS cells, seminoma cells, non-seminomas, and predominantly in spermatogonia in the hormone-treated groups. ALK4 and ActRIIB proteins were readily detected in CIS and seminoma cells, non-seminomas, as well as in spermatogonia and Sertoli cells in the hormone-treated samples. In striking contrast to the normal testis, ActRIIA protein was detected in a small proportion of seminoma cells, while expression was faint in non-seminomas and undetectable in CIS cells. Hormone-treated testis samples contained a higher proportion of cells positive for ActRIIA than control samples, with expression again detected predominantly in Sertoli cells and some spermatogonia. Differential expression of ActRIIA protein was verified using in situ hybridisation, which demonstrated ActRIIA transcripts within the same cell types. This suggests the testicular dysgenesis in both neoplastic (seminoma) and hormone-treated testis maybe linked with enhanced ActRIIA expression, highlighting the potential for activin to influence cell function in these testes.

ONTOGENY OF CAMP-DEPENDENT TYROSINE PHOSPHORYLATION-SIGNALING PATHWAYS DURING SPERMATOGENESIS AND EPIDIDYMAL MATURATION IN THE MOUSE
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Although the unique cAMP-dependent pathway correlated to sperm capacitation has been studied in depth during the post-ejaculatory events leading up to fertilization, little is known about when and where the capacity for tyrosine phosphorylation is activated during spermatogenesis and epididymal maturation. The study aimed to map the ontogeny of this signal transduction pathway during germ cell development and determine its association with the differentiation of a functional gamete. cAMP-induced tyrosine phosphorylation was not detectable until testicular germ cells had differentiated into spermatozoa. Entry of these cells into the epididymus was accompanied by the sudden activation of this pathway, initially in the principal piece of the cell and subsequently in the midpiece. In the caput and corpus epididymides the potential to express this pathway was inhibited by the presence of calcium in the extracellular medium. However calcium had no impact on the expression of this pathway in caudal epididymal cells presumably because the latter could regulate the intracellular concentration of this cation. The competence to phosphorylate the entire sperm tail, from neck to the tail-end piece, was accompanied by a capacity to exhibit hyperactivated motility on stimulation with cAMP. Tyrosine phosphorylation of proteins in the sperm head increased as spermatozoa entered the caput epididymis and then remained high until these cells entered the distal corpus and cauda. The proportion of cells exhibiting this pattern of tyrosine phosphorylation was not influenced by extracellular calcium or cAMP but was negatively correlated ($r^2 = 0.9$) with their competence to acrosome react; however this relationship did not appear to be causative. These studies indicate that the development of functional spermatozoa is accompanied by carefully orchestrated changes in tyrosine phosphorylation controlled by independent regulatory mechanisms in distinct subcellular compartments of these cells.

Figs: Immunofluorescence micrographs showing dynamic changes in the subcellular locations of phosphotyrosine expression after dbcAMP/PTX stimulation. Spermatozoa extracted from the testis (1) and in regions of caput (2), corpus (3) and caudal (4) epididymis. Scale bar = 5 µM.
PGP 9.5 AS A MARKER FOR GERM CELL DEVELOPMENT IN PRE-PUBERTAL AND IRRADIATED SHEEP TESTES.

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Quantifying germ cell numbers after irradiation or chemotherapy treatment allows assessment of future fertility. It is also necessary in recipient testes prior to germ cell transplant where endogenous germ cells should be minimized to improve transplant efficiency. In cattle, monkey and goat testes, the protein gene product (PGP) 9.5 has been used as a marker for spermatogonia providing a means of quantifying germ cell number, however the expression of PGP 9.5 during ovine testis development has not been previously reported and this study evaluates its use in the ovine.

The number of spermatogonia per tubule and per 100 Sertoli cells were counted using immunohistochemistry for a commercial available PGP9.5 antibody in ovine testis sections. Bovine sections were used as positive controls for PGP9.5 expression at similar development stages. Testis sections from bull calves (n=1) and ram lambs (n=3) were grouped into three maturity stages:

- < 2 months of age (gonocytes only)
- Pre pubertal 3-4 months of age (spermatogonia only)
- Pubertal (start of spermatogenesis)
- A 4th group of pubertal lambs (n=3) were irradiated (12 Gy dose) and the testes biopsied 3 weeks later to assess the effect on germ cell numbers.

<table>
<thead>
<tr>
<th>Testis stage</th>
<th>Ovine</th>
<th>BOVINE</th>
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<tr>
<td></td>
<td>Testis Wt (g)</td>
<td>Tubule Diameter (µm)</td>
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<tr>
<td>Gonocytes</td>
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<td>Single layer</td>
<td>30</td>
<td>107 ± 13 a</td>
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<tr>
<td>Spermatogenesis</td>
<td>110</td>
<td>167 ± 19 a</td>
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(One way analysis of variance P<0.05 for different subscripts within columns.)

PGP9.5 was expressed in ovine spermatogonia from single (pre-pubertal) and multiple layered tubules (pubertal). There was no detectable PGP 9.5 expression in ovine gonocytes (1 unstained gonocyte/cross section), which contrasts with bovine sections where PGP 9.5 is expressed in both gonocytes and spermatogonia. The number of PGP 9.5 positive spermatogonia cells increased as the testis developed and an irradiation dose of 12 Gy reduced PGP positive cells/100 sertoli cells from 36 to 4.

LONG-TERM EFFECTS ON THE TESTIS OF A SHORT PERIOD OF UNILATERAL CRYPTORCHIDISM IN RATS

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One testis of each of 4 adult rats were made cryptorchid for 48 h and both testes of 3 rats were heated at 43°C for 30 min. After 175 days, blood plasmas collected from testicular veins and posterior vena cava were analysed for testosterone by RIA, and the testes and isolated tubules fixed in Bouin's fluid for histological examination. Size of the previously cryptorchid testis fell to about 35% of controls). Only about 73% of the tubule cross-sections in the previously cryptorchid testes showed full spermatogenesis after 175 days. Of the affected tubules, about 35% contained no germ cells, while the most advanced cells were A spermatogonia in 35%, B gonia or preleptotene spermatocytes in 19%, leptotene-pachytenes spermatocytes in 4%, pachytenes in 9% and round spermatids in 3%. The corresponding figures for heated testes were 22, 26, 33, 7, 12 and 1%. Four heated testes from 2 rats had no tubules with full spermatogenesis, with less than 20% in the other heated rat. Testosterone levels in testis vein blood were higher in heated testes, but lower than control in testes which had been cryptorchid, while peripheral levels were lower in the cryptorchid rats but similar to controls after heating. After radiation, longterm disruption of spermatogenesis appears to be different, and has been attributed1 to spermatogonial arrest due to high testosterone levels and/or accumulation of interstitial extracellular fluid.

ASSESSMENT OF KOALA SPERM MITOCHONDRIAL FUNCTION WITH JC-1

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The fluorescent carbocyanine dye JC-1 has been used as a tool for evaluating mitochondrial membrane potential (MMP) in a range of cell types, including eutherian mammalian spermatozoa (1, 2 & 3) but not marsupial spermatozoa. The aim of this study was to establish a reliable protocol for the use of JC-1 in koala spermatozoa and then examine the effect of glycerol cytotoxicity, chilling to 5°C (-6°C/min) and a standard cryopreservation protocol (4) on MMP. Electro-ejaculates from 8 different koalas were obtained, immediately diluted 1:1 in Tris-citrate glucose (5) then treated and stained with JC-1 and Propidium Iodide (PI) as per manufacturer's instructions (http://probes.invitrogen.com/) in preparation for 3 experiments. Experiment 1 involved the addition of glycerol drop-wise to the semen sample at 35°C to a final concentration of 14% and incubated for 30 mins; glycerolated sperm were compared to sperm not exposed to glycerol. In experiment 2, diluted semen samples without glycerol were cooled to 5°C and then re-warmed to 35°C. For the final experiment, diluted koala spermatozoa were cooled to 5°C, glycerol added (14%) and then frozen at -6°C / min before being rapidly thawed and re-warmed to 35°C. MMP and PI status were assessed during these treatments by means of a cryomicroscope, which allowed direct observations at a range of temperatures (pre-freeze 35°C and 5°C; post-thaw 5°C, 20°C and 35°C). Table 1 summarises the results of all 3 experiments and indicates that (1) Koala sperm MMP and plasma membrane integrity were not significantly affected when exposed to 14% glycerol at 35°C; (2) chilling koala spermatozoa to 5oC also had no significant negative effect on MMP or PI; surprisingly, some dead (PI+) spermatozoa showed evidence of maintaining MMP. While cryopreservation of koala spermatozoa resulted in a predictable loss of MMP and membrane integrity, cryomicroscopy revealed that most pathology occurred during thawing.

Table 1: Mean percent gc (± SEM) spermatozoa (Pl+ or PI-) with high and low membrane potential (MMP) following exposure to 3 experimental treatments on a cryomicroscope.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>High MMP (Pl+)</th>
<th>Low MMP (Pl-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control - no glycerol</td>
<td>70 ± 8.0</td>
<td>3 ± 1.0</td>
</tr>
<tr>
<td>Glycerol (14%)</td>
<td>68 ± 5.5</td>
<td>4 ± 1.0</td>
</tr>
</tbody>
</table>

Experiment 1: Glycerol cytotoxicity

Control (35°C)                                     | 70 ± 8.0       | 3 ± 1.0       |
Cooled to 5°C and observed                        | 61 ± 9.2       | 4 ± 1.4       |
Re-warmed to 35°C and observed                    | 57 ± 8.9       | 6 ± 2.3       |

Experiment 2: Effect of Cold Shock

Control (35°C)                                     | 70 ± 8.0       | 3 ± 1.0       |
Cooled to 5°C and observed                        | 61 ± 9.2       | 4 ± 1.4       |
Re-warmed to 35°C and observed                    | 57 ± 8.9       | 6 ± 2.3       |

Experiment 3: Cryopreservation

Control (5°C)                                     | 70 ± 8.0       | 3 ± 1.0       |
Cooled to 5°C and observed                        | 61 ± 9.2       | 4 ± 1.4       |
Re-warmed to 35°C and observed                    | 57 ± 8.9       | 6 ± 2.3       |

Table 1: Mean percent gc (± SEM) spermatozoa (Pl+ or PI-) with high and low membrane potential (MMP) following exposure to 3 experimental treatments on a cryomicroscope.

ONE-SIDED EJACULATION OF SPERM BUNDLES IN THE ECHIDNA

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Observations of copulatory behaviour in the Prototheria are limited and there have been no descriptions of ejaculate characteristics from the taxa. We describe for the first time, an unusual form of behaviour (movie provided) of copulatory behaviour in the Prototheria are limited and there have been no descriptions of ejaculate characteristics from the taxa. We describe for the first time, an unusual form of behaviour (movie provided) of copulatory behaviour in the Prototheria. The ability to repeatedly access ejaculated semen from echidnas will facilitate a range of studies on sperm biology and the development of assisted breeding technology.

Figure 1: A - Erect short-beaked echidna penis demonstrating one-sided retraction of the right side of the glans penis (arrow) and pooled semen in the two rosettes of the functional left side; B and C - scanning and transmission electron micrographs of ejaculated sperm bundles (Scale bars - 10?m and 500 nm respectively); B - Apical view, C - Lateral aspect. c - "cement substance", Lg - left glans penis, R - rosette, Se - semen; Sp - sperm.

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THE OBJECTIVE ASSESSMENT OF PORCINE EPIDIDYMAL SPERM USING THE SPERM QUALITY ANALYZER IIb.

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The Sperm Quality Analyser IIb (Medical Electronic Systems, Migdal-Haemek, Israel) is an automated machine used widely to assess the fertility of males of a number of domestic species, and operates by detecting the scatter of light by sperm as an indicator of sperm motility. The present study evaluated systematically the SQA IIb as a means of analyzing objectively the motility of porcine epididymal sperm maintained in TALP-HEPES containing 3 mg/ml bovine serum albumin. Epididymal tissue of Landrace x Large white cross pigs was obtained from a local abattoir.

The serial dilution of a sperm sample with culture medium did not affect the sperm motility as judged manually (mean motility of 65.0 ± 4.4%). However, readings obtained with the SQA IIb for these diluted samples showed a significant (p<0.05) influence of sperm concentration on both the sperm motility and sperm motility index (SMI).

The manual and automated measurement of sperm motility was made for sperm suspended in bicarbonate-free medium at a set sperm concentration. The motility determined manually was seen to be good (55.0 ± 2.6%) but with a low level of progressive motility (1.7 ± 1.2%). The SQA IIb gave similar readings for motility (55.5 ± 3.4%) suggesting that the analyser recognised the non-progressive motility. The usefulness of the machine was then shown by demonstrating the toxicity of glycerol to sperm.

In summary, two major limitations of this machine were found, namely (i) the readings obtained are influenced by the concentration of the sperm suspension, and (ii) the machine finds difficulty in differentiating between progressive and non-progressive motility. However, if sperm concentration is held constant and one only requires an assessment of the total motility, then the machine can be used to detect objectively changes in sperm motility. This will be a boon to inexperienced workers such as students.

PROSTATE ATROPHY AND ABNORMAL EPITHELIAL CELL PROLIFERATION DUE TO TARGETED DISRUPTION OF THE PROSTATE EPITHELIAL ANDROGEN RECEPTOR IN PEARKO MICE

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2Austin Health, University of Melbourne, Melbourne, VIC, Australia

Stromal-epithelial interactions and androgen actions via androgen receptor (AR) within these compartments are important for prostate development and maturation. However, so far it has not been possible to clearly separate the roles of epithelial and stromal AR in mature prostate. We established a novel mouse model with targeted disruption of prostate epithelial AR to selectively study the role of epithelial AR in the mature prostate. A transgenic mouse line expressing Cre under a probasin promoter Pbns-cre were crossed with a mouse line containing a floxed AR gene, exon 3 flanked by loxP sites, to generate male progeny exhibiting a prostate epithelial specific AR knockout (PEARKO). Crossing Pbns-cre with ROSA reporter mice revealed Cre expression in epithelium of all prostate lobes, as well as seminal vesicle and epididymis epithelium at two weeks of age. At eight weeks of age, PEARKO males had significantly (p<0.05) decreased weight of prostate lobes (36, 47 and 80% of control, for anterior, dorsolateral and ventral prostate, respectively), but unchanged testis weight or serum testosterone compared with Cre littermate controls. Yet, despite decreased prostate lobe weights, microdissection revealed normal branching morphogenesis of all lobes in PEARKO mice. Volumetric proportion of epithelia was significantly (p<0.05) decreased and that of lumen increased in PEARKO anterior (44 and 39%, respectively) and dorsolateral (49 and 26%, respectively) prostate while the volumetric proportions of epithelia, lumen and stroma were similar for ventral prostate in PEARKO and control males. The most significant qualitative changes were abnormal clustering of epithelial cells predominantly in anterior lobe but also in dorsolateral and ventral lobes of PEARKO mice. These results indicate that disruption of epithelial AR signalling and thereby normal stromal-epithelial interactions lead to abnormal morphology and growth of prostate epithelial cells. (Academy of Finland #107825 and Finnish Cultural Foundation of Northern Savo)

ANDROSTANEDIOL AND DEVELOPMENT OF THE WOLFFIAN DUCTS IN TAMMARS

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Sexual differentiation in marsupials takes place after birth, when the young is developing in the pouch. Wolffian duct differentiation depends on androgens, but two questions are unanswered: which androgen drives this process, and how is the androgen delivered to the duct?

We have previously shown that 5a-androstanediol (5α-adiol; 5α-androstan-3α, 17β-diol) is the circulating androgen in developing males responsible for prostate and penile differentiation. Female tammar neonates treated with 5α-adiol from day
10 to day 35 retained their Wolffian ducts whilst the control treated females did not. Wolffian duct development in male neonates treated from day 10 to day 35 with 4MA, an inhibitor of 5α-reductase and DHT formation was inhibited. Unexpectedly 4MA treated males retained their Müllerian ducts. Müllerian ducts were also retained in oestrogen treated males in previous experiments, suggesting that inhibiting 5α-reductase may have allowed a build up of testosterone that was aromatised to oestradiol. We grafted neonatal testes beneath the skin of neonatal females and found that the Wolffian ducts in these females were retained, showing that testicular androgens do not need to be delivered locally to the duct, but can act via the systemic circulation.

Thus Wolffian duct development in this marsupial depends on a 5α-reduced steroid that can be delivered by the systemic circulation. The most likely candidate is 5α-androstanediol.

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**ANTI-ACTIVIN CONSEQUENCES OF GLUCOCORTICOID ACTION WITHIN THE MALE REPRODUCTIVE AXIS**

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Local activin actions are partly determined by follistatin (FS), inhibin and its co-receptor, betaglycan (BG). Inhibin binding to BG promotes its antagonism of activin actions through the sequestration of the type II receptors. Glucocorticoids are known to increase the expression of BG and FS in some non-reproductive tissues. Since gonadotrophs, Leydig cells and primary spermatocytes express glucocorticoid receptor (GR), we hypothesized that glucocorticoids inhibit activin actions in reproductive tissues through the up-regulation of BG and FS expression.

Mouse Leydig (TM3), gonadotroph (LβT2) and germ line [GC-2spd(ts)] cells, but not Sertoli (TM4) cells, were found to express GR, and all cell lines expressed BG and FS. Overnight treatment of these cells with the synthetic glucocorticoid, RU28362 (250 nM), gave the following tabulated changes (% of the matching vehicle control) in their relative BG and FS mRNA levels, and [125I]inhibin A binding to whole cells: [**, **P<0.05, P<0.01 relative to 100%]

<table>
<thead>
<tr>
<th>Cell line</th>
<th>BG mRNA level</th>
<th>FS mRNA level</th>
<th>[125I]inhibin A binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>TM3</td>
<td>258 ± 19**</td>
<td>500 ± 60**</td>
<td>134 ± 3**</td>
</tr>
<tr>
<td>TM4</td>
<td>113 ± 2*</td>
<td>81 ± 10</td>
<td>121 ± 10</td>
</tr>
<tr>
<td>LβT2</td>
<td>222 ± 23**</td>
<td>189 ± 40</td>
<td>126 ± 3**</td>
</tr>
<tr>
<td>GC-2spd(ts)</td>
<td>139 ± 9*</td>
<td>149 ± 17</td>
<td>133 ± 4*</td>
</tr>
</tbody>
</table>

As a representative cell line, TM3 cells were transfected with the activin-responsive pGRAS-luc reporter. Treatment of cells with RU28362 decreased the inhibin A IC50 for antagonism of activin A-stimulated luciferase expression from 190 to 77 pM.

In summary, glucocorticoid treatment increased BG expression, promoted its interaction with inhibin A, and stimulated follistatin expression in cell lines modelling gonadotrophs, Leydig cells and spermatocytes, but not Sertoli cells, consistent with the pattern of GR expression by these cell types. In TM3 cells, glucocorticoids concomitantly increased inhibin potency. Thus, glucocorticoids display dual anti-activin actions in multiple responsive cell types within the male reproductive axis.

Funded by the NH&MRC of Australia (RegKey 241000 & 198705)

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**CALMODULIN-DEPENDENT NUCLEAR IMPORT OF THE TESTIS-DETERMINING FACTOR SRY**

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Sex determination in mammals is determined by the chromatin-remodeling factor, SRY (Sex determining Region on the Y chromosome). SRY is expressed embryonically in Sertoli cell precursors and, through its high mobility group (HMG)-box domain, acts as a switch within the nucleus at specific DNA targets to modulate gene expression, leading to the development of the testis. A number of mutations in SRY that result in human XY sex reversal map to one of SRY’s two independently acting nuclear localisation signals (NLSs) that flank its DNA binding domain. The C-terminal NLS (C-NLS) targets SRY to the nucleus through the conventional nuclear import receptor importin-β1 (Imp-β1), but no importin has been shown to bind the N-terminal NLS (N-NLS), although it is known to interact with the Ca<sup>2+</sup>-binding protein calmodulin (CaM). In this study, we examine various missense mutations in the SRY N-NLS from XY sex-reversed females for effects on nuclear import and ability to interact with CaM and Imp-β1. The mutations were all found to result in reduced nuclear localisation in transfected cells compared to wild type. The CaM antagonist, calmidazolium chloride (CDZ), was found to significantly reduce SRY nuclear accumulation, indicating dependence of SRY nuclear import on CaM. Intriguingly, N-NLS mutants were resistant to CDZ’s effects, implying a loss of interaction with CaM, which was confirmed directly by in vitro binding experiments. These results strongly implicate a CaM-dependent nuclear import pathway for SRY mediated by the N-NLS that, together with the C-NLS, is required to achieve threshold levels of SRY in the nucleus for male sex determination during foetal development.
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LIVER RECEPTOR HOMOLOGUE-1 (LRH-1) REGULATED GENES WITHIN THE TESTIS.
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LRH-1 is an orphan nuclear receptor localised in the Leydig and germ cells. It has been associated with regulating cell proliferation and tumourgenesis, but its function within the testis has yet to be elucidated. We have demonstrated that LRH-1 has the ability to regulate aromatase within cells isolated from adult rat testes, however little is known about other LRH-1 regulated genes. Therefore we aimed to identify potential LRH-1 regulated genes by two complementary methods. Affymetrix microarrays and a database of proximal promoters (PAGEN@UIC) were utilised to ensure the genes of interest are both potentially regulated by LRH-1 and possess the LRH-1 consensus DNA binding sequence (CAAGGGTCA). Affymetrix microarray analysis was performed on primary Leydig and germ cells infected with a full length LRH-1 recombinant adenoviral construct to over express LRH-1, to determine genes that are either up or down regulated in the presence of LRH-1. The Affymetrix microarray analysis generated a list of 200 genes for each cell type that were regulated by LRH-1. Nine genes were selected and identified to contain the consensus LRH-1 DNA binding site within their promoter region (SOCS1, BMP4, IGFBP5, FABP9, Wnt1, Foxa2, tsag13, Gdf10, IL6, Klf5 and Kit ligand). There were five other genes of interest regulated by LRH-1 which did not appear to contain the consensus sequence in their promoter region, but may be indirectly regulated by LRH-1. Genes which did not appear to be regulated by LRH-1, but contained the consensus LRH-1 sequence were also identified as they may potentially be important. Many of the identified genes are known to be involved in cell cycle and development and they may have important, but as yet unknown roles in the testis. Work is currently being performed to validate the selected genes within the different cells and to determine their role in testicular physiology.

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ACTIVIN βC-SUBUNIT IS A REGULATOR OF TESTIS AND LIVER FUNCTION: IMPLICATIONS FOR ACTIVIN BIOLOGY
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Dimeric activins (βA βA , βB βB) are growth and differentiation factors with potent in vivo activities in diverse biological systems including mesoderm induction and early embryogenesis. The related activin βC subunit forms homodimers as well as heterodimers with βA and βB subunits in vitro, but appears to have little functional significance based on lack of abnormalities in activin βC-subunit knock-out mice. We proposed the activin βC-subunit is a functional antagonist of activin A, forming heterodimers in tissues that co-express other activin subunits. To test this hypothesis we generated activin βC-subunit over-expressing mouse lines and observed pathologies in testis and liver. Activin βC-subunit over-expression reduces circulating activin A levels and male transgenic mice develop a progressive, age-related, decrease in fertility. A decline in litter sizes was associated with reduced sperm output due to a stage-specific increase (stages V-VIII and IX-XI) in apoptosis during spermatogenesis and impaired sperm motility. Further, the livers of transgenic mice were enlarged due to an imbalance between hepatocyte proliferation and apoptosis; foci of inflammatory cells were evident which were associated with significant changes in liver enzymes. The data demonstrate that in tissues in which activin βC-subunit expression is up-regulated, activin biology is impaired leading to pathologies as demonstrated in the liver and testis. Collectively our data suggest the activin βC-subunit is a novel regulator of activin bioavailability. These data have implications for activin biology in any tissue in which βA and βC-subunits are co-expressed.

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GLY COSYLATED FORMS OF HUMAN INHIBIN A AND B SHOW MARKED DIFFERENCES IN IN VITRO BIOACTIVITY
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Inhibin A and B are dimeric gonadal proteins consisting of an α and either βA or βB subunits. In contrast to inhibitin A, the structure function characteristics of inhibin B are poorly described. The aim of this study was to purify and characterise glycosylated 31 and 34k forms of recombinant human inhibitin A and inhibin B. Inhibitin A and B were purified from conditioned culture medium using anti-inhibitin α subunit immunofinity chromatography and RP-HPLC. The masses of the purified inhibitin preparations were determined by specific inhibitin A and B ELISAs and their in vitro bioactivities determined by in vitro bioassay based on FSH suppression in rat pituitary cells in culture. The specific bioactivities (expressed as a ratio of in vitro bioactivity to immunoactivity; B/I ratio) of inhibitin A and B and their glycosylated forms were then determined. The mono-glycosylated 31k inhibitin A was 4.5 times more potent than the di-glycosylated 34k inhibitin A, (B/I ratio 1.18±0.16 (mean±sd, n=3-8) vs 0.25±0.05, p<0.001, resp). Deglycosylation of 31k inhibitin A resulted in a B/I ratio of 2.75±0.58.
suggested that glycans play an inhibitory role in their biological activities. Similarly, the 31k inhibin B was significantly (p<0.001) more potent (0.91±0.21) than the 34k form (0.51±0.1). However, de-glycosylation of the 31k inhibin B showed a decrease (0.54±0.10) in B/I ratio. Note that the bioactivities of inhibin A and B were broadly similar. It is concluded that the 31k and 34k mol wt forms of both inhibin A and B differ in their bioactivities and that human inhibin preparations with differing proportions of the 31 and 34k inhibin forms will show differences in function. These findings may have implications in the physiology of inhibin if the proportions of the 31/34k inhibin forms produced are regulated.

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IN VIVO REGULATION OF TIGHT JUNCTION PROTEINS BY GONADOTROPHINS IN THE ADULT DJUNGARIAN HAMSTER TESTIS
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The blood-testis barrier (BTB), formed by binding between tight junction (TJ) proteins (occludin, claudin-11, claudin-3 and junction adhesion molecule) between Sertoli cells is essential for spermatogenesis. This study aimed to assess the hormonal regulation of testicular TJ proteins in vivo using the adult Djungarian hamster, in which gonadotrophins and spermatogenesis cycle naturally between normal and depleted states. Long day (LD) photoperiod (16L:8D) adult hamsters were exposed to short day (SD) photoperiod (8L:16D) for 11 weeks to suppress gonadotrophins and spermatogenesis and then received 6 I.U FSH (2-10 days) to re-initiate spermatogenesis. Testes were Bouin's fixed for immunohistochemistry (claudin-3, claudin-11 and occludin) or had RNA extracted for TJ mRNA quantitation by real-time PCR.

Claudin-11 and occludin in the LD hamster were localised to the basal aspect of Sertoli cells consistent with the BTB. TJ proteins were disorganised in the SD hamster and localised principally to Sertoli cell cytoplasm. After 2 days of FSH replacement TJ proteins were reorganised, and resembled the LD phenotype by 10 days. Claudin-3 (expressed in forming TJs) was not expressed in LD Sertoli cells but was localised to apical cytoplasm and around germ cells in SD animals. After two days of FSH treatment, intense claudin-3 reactivity localised to basal aspects of Sertoli cells, consistent with forming BTB. Immunoactivity progressively decreased by 10 days treatment. In contrast claudin-11 was maximal and resembled the LD phenotype at this time. Compared to LD hamsters, TJ mRNA levels (claudin-3, claudin-11, occludin) were increased (2 fold, p<0.05) in SD animals where the BTB is known to be non-functional.

We conclude that testicular TJs are regulated by FSH in the Djungarian hamster, as evidenced by the disorganisation of TJ protein in the SD animal and reorganisation after FSH treatment. High TJ mRNA levels and immunoactivity in the SD hamster suggest that poorly organised TJ proteins are still present but not functional. The initiation of claudin-3 expression after gonadotrophin suppression and transient expression during BTB formation supports the hypothesis that claudin-3 is a component of newly forming TJ in the testis.

(2) Tarulli et al 2006 Biol Reprod 74:798
(4) Meng et al 2005 PNAS 15:16696

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GENE EXPRESSION PROFILING BY MICROARRAY ANALYSES OF BOVINE GRANULOSA CELLS FROM SMALL AND LARGE HEALTHY ANTRAL FOLLICLES
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From primordial to ovulatory stage, ovarian follicles undergo a number of maturational changes, eventually leading to ovulation or atresia. During the later stages of antral follicle development (follicle diameter >5 mm), a specialized basal lamina-like matrix, foci matrix, develops between the granulosa cells (Matrix Biol. 2004 23:207-17). We hypothesise that foci matrix plays an important role in the maturation of granulosa cells in the days leading to ovulation. To identify differentially expressed genes in bovine granulosa cells (GC) before and after foci matrix appearance, we collected pairs of bovine ovaries from a local abattoir and dissected small (≤ 5 mm) and large (>12 mm) antral follicles. GCs of these follicles were harvested and immediately frozen at –80C for subsequent RNA isolation. Atretic follicles were excluded on the basis of histological examination of a biopsy of the follicle wall. GC isolates with minimal thecal contamination (< 1.0% of CYP17 mRNA levels normally observed in thecal layers) were used. RNA preparations of GCs of small (n = 5) and large (n = 4; equal levels of CYP19 mRNA) follicles were then hybridised to bovine Affymetrix GeneChips by the Australian Genome Research Facility. Analyses were conducted using the Bioconductor software package (www.bioconductor.org). As expected, a large number of genes (1600 genes at P < 0.01) were differentially expressed between large and small follicles. Several of these have been well characterized previously at different stages of follicle development. However, many of the identified genes were not previously known to be involved in follicular development. The results of our study will provide new insights into the regulation of follicle maturation and provide a basis for ongoing and future research addressing follicle development.
CORRELATIVE GENE EXPRESSION AND FOLLICULAR DOMINANCE IN THE BOVINE
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Focimatrix is extracellular matrix protein of the basal lamina type containing collagen, laminin, nidogen and perlecan (1). It is deposited between granulosa cells of bovine follicles > 5 mm in diameter (1). Very little is known about what regulates the deposition of focimatrix, or its role(s). Therefore its expression was measured in dominant and subordinate follicles relative to other indices of granulosa cell maturation. Pairs of ovaries were collected at an abattoir from cycling, non-pregnant cows (n = 30) and the three largest follicles were harvested per cow (8-18 mm). A section of the follicle wall was frozen in OCT compound for immunohistochemistry and only healthy follicles were examined (n = 44). Granulosa cells were scraped from the remaining portion of the follicle, and frozen for subsequent RNA isolation and qRT-PCR analyses. Focimatrix within the membrana granulosa was quantified by image analysis (NIH Image) from sections immunostained with an antibody to laminin 111 (EHS). Dominant follicles had significantly increased mRNA for steroidogenic enzymes and the LH receptor in comparison to subordinate follicles. Focimatrix content, and mRNA for nidogen-2 were also significantly greater in dominant follicles (P < 0.05). Furthermore, comparison of dominant follicles of equivalent size from cows with either healthy or atretic subordinate follicles revealed significantly higher levels of mRNA for nidogen-2 and LHR when subordinate follicles were atretic (P < 0.05). Multiple correlation analysis identified the following groupings of genes which appeared to be co-ordinately regulated; (A) FSHR and INHA (P = 0.085), INHBB and nidogen-2, (B) LHR and 3bHSD, and (C) laminin b2, collagen type IV a1, perlecan, CYP11A1 and CYP19. Levels of mRNA for the LH receptor, 3bHSD, CYP19, nidogen-2 and focimatrix correlated positively with follicle diameter. Taken together, these results suggest a causal relationship between extracellular matrix production by granulosa cells and oestrogen and progesterone synthesis.


MECHANISM BY WHICH FGF9 STIMULATES OVARIAN PROGESTERONE PRODUCTION
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FGF9, a member of the fibroblast growth factor family (FGF) is present in the ovary (1). We have shown previously that FGF9 stimulates granulosa cell progesterone production (2), although the actual mechanism has yet to be elucidated and that FGF9 protein is present in corpora lutea suggesting that it may act as a luteinisation factor. In these studies we investigated: 1) the impact of FGF9 on the expression of steroidogenic enzymes by granulosa cells (GC) and 2) an effect of LH, on FGF9-stimulated progesterone production.

GC from 21 day old diethylstilboestrol-treated rats were cultured for either 16h (RNA) or 1 day with FSH (100ng/ml) to stimulate LH receptors, followed by a media change and 2 days in McCoy's 5C with FGF9 (0.1 and 50ng/ml) ± LH (100ng/ml), ± FSH (100ng/ml). Progesterone was measured by radioimmunoassay. GC expression of P450 side chain cleavage (SCC), steroidogenic acute regulatory protein (StAR), 3β hydroxysteroid dehydrogenase (3βHSD) and GAPDH were measured by PCR.

SCC and 3βHSD mRNA expression was stimulated by FGF9 (10-, 3-fold respectively), but not to the same extent as FSH alone (15-35-fold). In contrast, StAR mRNA expression was stimulated by FGF9 beyond that of FSH alone (1.8-fold), with FGF9/FSH treatment further enhancing the expression (1.7-fold). LH treatment had no effect on FGF9-stimulated GC progesterone production; although in the presence of FSH an enhanced response was observed. These data suggest that FGF9 acts via StAR to stimulate GC progesterone production and that FSH stimulation is more important than LH in this pathway. Supported by the NH&MRC of Australia (Regkey 241000 & 198705)


THE LOCALISATION OF CYCLIN B1 AND FAT FACETS IN MOUSE (FAM) IN MURINE OOCYTES UNDERGOING MATURATION IN VITRO
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During meiosis, maturation promoting factor (MPF) activity increases to arrest at metaphase I (MI). MPF is a serine/threonine kinase heterodimer composed of cdc2 and cyclin B1. The ubiquitin-proteasome pathway (UPP) regulates MPF activity by degrading cyclin B1 causing MPF levels to fall and meiosis to proceed. We showed that the proteasome 'core' moves to the spindle after germlinal vesicle breakdown (GVBD) and remains there. This study reports the localisation of cyclin B1 and a deubiquitinating enzyme, fat facets in mouse (FAM or USP9x), which was implicated in chromosome alignment during

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mitosis in HeLa cells (Vong et al., 2005). Confocal immunofluorescence microscopy was used to localise cyclin B1 and FAM in mouse oocytes undergoing maturation in vitro. Prior to maturation, cyclin B1 was localised within the germinal vesicle (GV) whilst FAM was found in the cytoplasm. However upon GV break down (GVBD), cyclin B1 and FAM were both localised to the developing meiotic spindle where they remained during MI and MII. During anaphase-telophase I (AI/TI), both were found between the separating homologous chromosomes. The co-localisation of FAM, cyclin B1 and the 20S proteasome after GVBD suggests that together with the MAP kinase/P90rsk pathway, FAM may participate in regulating levels of cyclin B1 during the AI/TI transition by antagonising the UPP via deubiquitination of cyclin B1. Alternatively, as in mitosis, FAM may regulate chromosome alignment and segregation in meiosis similarly to the finding in mitosis (Vong et al., 2005).


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IMPROVED DEVELOPMENT OF MURINE EMBRYOS DERIVED FROM COCS MATURED WITH THE O-LINKED GLYCOSYLATION INHIBITOR, BADGP.

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The hexosamine biosynthesis pathway is an alternate fate for glucose metabolism and is significantly up regulated in the presence of glucosamine (GlcN), a popular dietary supplement. Previously, we have shown that the addition of GlcN into cumulus oocyte complex (COC) maturation media impairs embryo development. Hexosamine biosynthesis up regulation is known to increase O-linked glycosylation of intracellular signalling molecules. In this study we assessed the impact of in vitro maturation (IVM) of COCs in the presence of GlcN and BADGP (benyl-2-acetoamido-2-deoxy-a-D-galactopyranoside, in 2% methanol), an inhibitor of O-linked glycosylation, on subsequent embryo development. COCs were collected from 21 day old mice and matured (100 mIU/COC) in media containing 5 mM glucose under various conditions prior to fertilization and culture: 1) 0 mM GlcN - 2% methanol; 2) 0 mM GlcN + 2% methanol 3) 2.5 mM GlcN - 2% methanol; 4) 2.5 mM GlcN + 2% methanol; 5) 0 mM GlcN + 1 mM BADGP; 6) 2.5 mM GlcN + 1mM BADGP or 7) 2.5 mM GlcN + 2mM BADGP. Embryo development was not influenced by the inclusion of methanol in IVM media. Rates of blastocyst development were reduced following the maturation of COCs with 2.5 mM GlcN. Addition of either 1 mM or 2 mM BADGP to maturation media containing 2.5 mM GlcN increased blastocyst development rates (57% and 52% respectively) relative to media containing 2.5 mM GlcN (25%, P<0.01). We conclude that a contributing component of the reduced embryo viability occurring after exposure of COCs to glucosamine is the up regulation of O-linked glycosylation of signalling molecules during oocyte maturation.

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FETAL AND PLACENTAL OUTCOMES ARE PROGRAMMED BY OXYGEN CONCENTRATION DURING MATURATION OF MURINE OOCYTES.

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Although pO2 of the ovarian follicle is thought to influence oocyte developmental competence, the impact of differing pO2 levels during oocyte maturation (IVM) on subsequent developmental outcomes has not been explored. Previously, we found that pO2 (either 2, 5, 10 or 20% O2, 6% CO2) during IVM of murine cumulus oocyte complexes (COC) had no effect on maturation rate or subsequent fertilisation, cleavage and blastocyst development rates [Banwell et al, Proc. SRB 2005:233]. However, 2% O2 results in blastocysts with higher (P<0.05) trophectoderm cell number when compared to 20%, with 5 and 10% O2 yielding intermediate cell numbers. This study investigated the effect of pO2 during IVM on post-transfer outcomes. Immature COCs were collected from the ovaries of eCG-stimulated B6CBAF1 females (21 d) and cultured for 17-18 h under 2, 5 or 20% O2. In vivo matured COCs were also collected post-hCG. After IVF/IC (under 5% O2), 6 blastocysts were transferred to each uterine horn of pseudopregnant Swiss recipients. Fetal and placental parameters were measured on day 18 of pregnancy. The ability of embryos to implant or develop was not altered by pO2 during IVM. However, fetal and placental weights were reduced (P<0.001) in the 5% O2 group (823.3 ± 28.1 mg and 87.4 ± 4.0 mg respectively) compared to the 2% (870.2 ± 26.7 and 98.7 ± 5.5), 20% (928.5 ± 26.1 and 100.1 ± 5.5) and in vivo matured groups (879.3 ± 32.3 and 104.5 ± 5.4). In contrast, the fetal:placental weight ratio was highest in the 5% O2 treatment, suggesting these placenta, whilst small compared to other treatment groups, are still efficient. This is the first evidence that programming of fetal/placental growth occurs from physiologically relevant treatments applied during oocyte maturation. Further evidence implies that a metabolic basis underpins such developmental programming.
CHANGES IN OVARIAN GENE EXPRESSION AND MAMMARY DEVELOPMENT IN THE BMAL1 KNOCKOUT MOUSE

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There is strong epidemiological evidence indicating that disruption of endogenous circadian rhythms causes a range of health problems ranging from metabolic and cardiovascular disorders to infertility and poor reproductive function. Circadian rhythms are generated by a suite of "clock gene" transcription factors that are cyclically expressed in the brain and peripheral tissues. The CLOCK and BMAL1 transcription factors regulate the expression of genes involved in cell growth, angiogenesis and development. The Bmal1 knockout mouse provides a model to analyse the impact of the loss of circadian rhythms on reproductive physiology at the molecular level.

The ovary has not previously been evaluated for cyclic expression of "clock genes" across 24 hours. Here we show that the "clock genes" Bmal1 and Per2 being expressed cyclically across 24 hours (with a 5-fold and 3-fold change respectively). Interestingly, other transcription factors including RevErba (9-fold change) and albumin D-element binding protein (DBP)(6.5-fold change) are also rhythmically expressed. Analysis of gene expression in the ovary of Bmal1 knockout mice has revealed the genes Per2, RevErba and DBP are constitutively (but not rhythmic) expressed. These genes are either chronically upregulated (Per2 185% of WT) or downregulated (12% and 34% of WT, for RevErba and DBP respectively).

Further to previous evidence of poor pubertal development in the Bmal1 knockout mouse, mammary gland development in Bmal1 knockout females is compromised, with 2 month old females showing a 38% reduction in ductal formation and a 60% reduction in ductal branching.

The loss of circadian rhythms by removal of Bmal1 causes significant perturbation in mouse fertility. The observation that ovarian gene expression is disrupted by the loss of Bmal1 poses the further question of how these transcriptional changes are effecting ovarian function.

KALLIKREIN 4 EXPRESSION IN MOUSE OVARIIES WITH SEROUS INCLUSION CYSTS.

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Epithelial ovarian cancer (EOC) remains a lethal disease because few specific biomarkers exist for early stage detection. Many serous EOCs arise from fluid-filled "inclusion" cysts in the ovary, lined with cells from ovarian surface epithelium (OSE). Kallikrein serine protease 4 (KLK4) is poorly expressed in OSE, but up regulated in serous EOC cell lines and tumours and is thus a potential biomarker for serous EOC. Mouse ovaries subjected to incessant ovulation form ovarian inclusion cysts which are similar histologically to human benign serous cystadenomas. We investigated Klk4 protein expression in mouse ovaries with high lifetime ovulation number, to determine if Klk4 protein was up-regulated during inclusion cyst development and whether Klk4 expression correlated with histopathology in the mouse ovary.

Incessant ovulation was induced for up to 12-months by keeping Swiss Webster mice in cages divided by a screen. Breeding stock of the same age were controls. Mouse Klk4 expression was measured by immunohistochemistry with three anti-human KLK4 anti-peptide antibodies, directed to the N-terminus (Ab01), mid region (Ab03) and C-terminus (Ab05) of human KLK4. Cytoplasmic immunoreactivity was detected with Ab01 and Ab03 and nuclear immunoreactivity with Ab05, in stromal cells, luteal cells, granulosa cells of some apoptotic antral follicles and in some, but not all oocytes in primordial or primary follicles, in ovaries from incessantly ovulated mice or breeders. No immunoreactivity was detected in OSE. Cysts were observed in ovaries from both breeders and incessantly ovulated mice. Immunoreactivity with Ab01/03 was inconsistent in cyst cells, although some dysplastic cells showed basal cytoplasmic staining with Ab03. Strong nuclear staining was observed with Ab05 in the majority of cyst cells, with weaker staining in rete ovarii. We conclude from these preliminary studies that Klk4 expression is up-regulated in inclusion cysts observed in cystic mouse ovaries.
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EXPRESSION OF SERINE PROTEASE HTRA3 DURING OVARIAN DEVELOPMENT AND FOLLICULOGENESIS IN THE RAT

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We identified a high temperature requirement factor A serine protease (Htra3) in the mouse ovary. Mouse Htra3 exists in two isoforms due to alternative splicing (long and short), and is highly identical to rat Htra3 (mRNA: 93%, protein: 97%). The aim of this study was to examine the expression of Htra3 in the rat ovary during ovarian development.

Real time reverse transcription-PCR (RT-PCR) measured the expression of Htra3 mRNA in days(d) 4, 8, 12 and 100 (adult) rat ovaries after birth (n=3 for each time point, each 3 samples of d4, 8 and 12 were pools of 20-40 ovaries, all samples measured against GAPDH). The levels of long Htra3 significantly increased from immature, d4, 8 and 12, to d100 rat ovaries. The levels of short Htra3 were not significantly different between the age groups.

Table 1. Relative Concentration (fg) of Htra3 mRNA During Rat Ovarian Development

<table>
<thead>
<tr>
<th>Days after birth</th>
<th>4</th>
<th>8</th>
<th>12</th>
<th>100 (adult)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long</td>
<td>0.89 ± 0.38</td>
<td>0.98 ± 0.55</td>
<td>14.29 ± 4.29</td>
<td>46.71 ± 11.7</td>
</tr>
<tr>
<td>Short</td>
<td>0.44 ± 0.18</td>
<td>0.18 ± 0.07</td>
<td>0.22 ± 0.07</td>
<td>0.56 ± 0.20</td>
</tr>
</tbody>
</table>

Compared to d100, *P<0.01, †P<0.05

Htra3 protein was localised by immunohistochemistry to large round interstitial cells in d4 and 12 ovaries (n=12 ovaries). In adult ovary, moderate staining for Htra3 protein was observed in interstitial cells and cumulus granulosa cells whilst intense staining was seen in large round luteinising cells of the corpus luteum.

These findings suggest a role for Htra3 in ovarian development, folliculogenesis and particularly in the process of luteinisation.

We next plan to confirm the association between Htra3 expression and luteinisation in a cell culture model. Granulosa cells isolated from diethylstilboestrol treated immature rats (21 days old) will be treated with follicle stimulating hormone (FSH) to induce luteinisation in primary culture and the levels of Htra3 mRNA and protein will be measured.

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(1) Nie, G. et al., 2003. Molecular Human Reproduction 9, 279-290

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USE OF A SINGLE-FOLLICLE-WAVE CYCLE TO STUDY ACUTE EFFECTS OF CHANGES IN NUTRITION ON OVULATION RATE IN EWES

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Nutritional supplements fed for short periods, 4-12 days before luteolysis, increase ovulation rate in sheep, but the response is unreliable (1,2). To control variance caused by interactions between the timing of the supplement and the stage of follicle development, we developed a "one-wave cycle" in which follicular waves are synchronised among ewes. We then used this model to test supplements that were expected to enhance ovulation rate. In two experiments, Merino ewes received 3 injections of 250 ug progesterone at intervals of 7 days to synchronise their follicle waves. Nutritional treatments were applied for 6 days, from 2 days after the second until the third injection. Ovulation rate was measured by transrectal ultrasonography (3) 10-14 days later. Experiment 1 used 110 ewes (age 4-5 years, live weight 50 kg, condition score 3.6) that were fed a maintenance diet from the first prostaglandin injection. Supplemented ewes (n = 55) were offered 500 g lupins per head per day for the 6-day treatment period. Experiment 2 used three groups of 80 ewes (age 6 years, live weight 60 kg, condition score 3.8) that were grazing dry summer pasture. One of the groups was offered 460 g Kasper peas and another was offered 500 g lupins per head per day for the 6-day treatment period. In both experiments, the one-wave model was successful with all ewes ovulating synchronously. In Experiment 1, the ovulation rate was increased by 14% higher in the ewes fed lupins (1.55 ± 0.06 vs 1.36 ± 0.07, P = 0.06). In Experiment 2, the ovulation rate for the ewes fed lupins was 10% higher than in the controls (1.48 ± 0.06 vs 1.35 ± 0.06, P = 0.06). For the ewes fed Kasper peas, it was similar to that of the controls (1.31 ± 0.06). We conclude that a 'cycle' that is restricted to a single follicle wave can be used to study the effect of short-term nutritional supplements around the time of follicle selection on ovulation rate in ewes. Current studies are determining the impact of this approach on fertility and embryo survival.

IDENTIFICATION OF GENES IN HUMAN ENDOMETRIAL AND STROMAL CELLS THAT ALTER DURING THE AQUISITION OF RECEPTIVITY

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The human embryo can implant into the maternal endometrium only during the window of implantation, a limited time span (days LH+5-10) when the endometrium is receptive. The receptive phase is accomplished by molecular changes in the endometrial epithelial (EEC) and stromal cells (ESC), but these are not well defined. The aim of this study was to identify genes expressed in human endometrial epithelial and stromal cells during the window of implantation. Endometrial biopsies were collected from women (N=5) at days LH+3 and LH+7 during the same menstrual cycle. EEC and ESC were separated by mechanical and enzymatic procedures and total RNA was extracted to determine differentially expressed genes using the Affymetrix GeneChip HG-U133 plus 2.0 microarrays. During the transition from LH+3 to LH+7, 466 genes were upregulated and 443 genes were downregulated in EEC, whereas 402 genes were upregulated and 267 genes were downregulated in ESC. The genes with the largest increased fold change were: chemokine (C-X-C motif) ligand 14 (927.8 fold) and progestagen associated endometrial protein (608.1 fold) for EEC, and for the ESC were progestagen associated endometrial protein (1990 fold) and solute carrier family 15 member 1 (89.4 fold). The most downregulated genes were transmembrane protein 16C (45 fold) and P21(CDKN1A)-activated kinase 7 (39 fold) for EEC, and microfibrillar-associated protein 3-like (30.7 fold) and secreted frizzled-related protein 4 (20.2 fold) for ESC. Differentially expressed transcripts for interleukin-1beta (IL-1B), complement component 3, progestagen associated endometrial protein and chemokine (CXC motif) ligand were confirmed by Real-Time RT-PCR. The data has identified genes differentially expressed by EEC and ESC in the receptive compared to non-receptive endometrium. Some of these genes may be a tool for identifying genes involved in decidual function and may be involved in infertility.

ACTIVIN INHIBITORS RETARD HUMAN ENDOMETRIAL STROMAL CELL DECIDUALISATION

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Human endometrial stromal cell (hESC) decidualisation is crucial for successful implantation and placental development. Activin A, a locally produced TGF beta superfamily member, promotes hESC decidualization in vitro (1). Activin inhibitors, SB431542 (an activin type I receptor inhibitor) and M108A (a novel activin type II receptor antagonist) block activin signalling in different cell systems in vitro (2,3). Other TGF beta superfamily members that utilise these receptors with differing affinities may also be inhibited. SB431542 potentially inhibits TGF beta -1,-2,-3; activins; GDF-8 and Nodal. M108A potentially inhibits activins; BMP-2,-4,-7; GDF-5,-8,-11 and Nodal. We postulated that blocking activins with either SB431542 or M108A would reduce hESC decidualisation. This study aimed to determine both the expression of TGF beta superfamily members and the effect of SB431542 and M108A in hESC during decidualisation. Decidualisation was assessed using an in vitro model in which combinations of cAMP, SB431542 (1.25-10µM) or M108A (0.375-25nM) were administered to hESC for 4-5 days. Medium was collected for prolactin (PRL) assay (a decidual marker). RNA was isolated from decidualised hESCs and ligand mRNA expression was analysed by RT-PCR. Ligand protein in decidual cells was determined by immunohistochemical analysis. Prolactin secretion from hESC was reduced following addition of either SB431542 or M108A. Activin- b A,- b B; BMP-2,-4,-7; GDF-5,-8,-11 mRNA were expressed by decidualised hESC in vitro. A ctitin b A,- b B, GDF-5 and -8 immunoreactivity was evident in decidual cells in human endometrium in vivo, suggesting a possible involvement of these family members in addition to activins in decidualisation. The reduction in prolactin observed with both inhibitors suggests activins are important in driving decidualisation. Further studies are required to elucidate the role of GDF-5 and GDF-8 in decidualisation. This study highlights the importance of activins during decidualisation, and that their manipulation may be used for future developments in human infertility.

FLUID TRANSPORT IN THE RAT UTERUS DURING EARLY PREGNANCY

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Implantation of the rat blastocyst is a highly regulated process, involving conversion of the uterine environment into one which is receptive to an implanting blastocyst. Part of this process involves a change in the fluid dynamics of the uterus during implantation, going from a lumen full of fluid on day 1 of pregnancy to close apposition between the luminal epithelium and trophoblastic cells at the time of implantation. Currently mechanisms regulating this change in luminal fluid volume are unknown. Aquaporins, a family of transmembrane water channels, are involved in the regulation of water movement across epithelial barriers. We investigated several aquaporins in the rat uterus during early pregnancy using reverse transcriptase PCR and found the presence of AQP1, AQP5, AQP7, AQP8 and AQP9. Immunofluorescence and immunogold electron microscopy techniques were then used to investigate the localisation of these aquaporin isoforms in the uterus during early pregnancy. AQP1 was localised to the myometrium and was shown to increase at the time of implantation while AQP5 was seen in the apical plasma membrane of luminal epithelial cells at the time of implantation. Both AQP5 and AQP9 were localised to the glandular epithelial cells, with AQP5 appearing at the time of implantation. Fluid reabsorption at the time of implantation thus may be mediated by AQP5 channels in the apical plasma membrane of luminal and glandular epithelial cells. It is also likely that AQP9 plays a role in reabsorption of glandular fluid. These results also suggest that AQP1 in the myometrium could facilitate fluid absorption into the muscle leading to swelling and mechanically closing down the uterine lumen around an implanting blastocyst.

EVOLUTION OF VIVIPARITY IN REPTILES: ROLE OF TIGHT JUNCTIONS OF THE UTERINE EPITHELIUM

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Evolution of viviparity involves the transition from egg-laying to live-bearing and is associated with changes in the functional role of the uterine epithelium. In oviparous amniote vertebrates, the uterine epithelium protects the developing egg, whereas in viviparous species the uterine epithelium becomes a placenta with some nutritive function. This study focuses on the tight junction of uterine epithelium as a key indicator of changes in uterine function with the evolution of viviparity. Tight junctions between adjacent cells constitute the barrier to the passage of ions and molecules through the paracellular pathway and functions as a ‘fence’ within the plasma membrane to create and maintain apical and basolateral membrane domains and can thus be expected to be more developed in viviparous species.

Lizards, Saiphos equalis and Lerista bougainvillii that exhibit bimodal reproduction provided excellent models for direct comparison between uterine epithelium of oviparous and viviparous species. A viviparous lizard with a relatively complex placenta, Pseudemoia entrecasteauxii, was also used to establish whether placental complexity is correlated with a more complex tight junctional structure in the uterine epithelium.

Uterine epithelium from lizards was analysed using routine transmission electron microscopy (TEM). Immunohistochemical analysis of occludin, an integral protein that exclusively labels tight junction strands was carried in conjunction with western blots. Up-regulation of occludin indicated increased numbers of tight junctional strands and therefore a tighter regulation of the paracellular pathway.

Tight junctions were, for the first time, identified in oviparous and viviparous lizards ultrastructurally, but occludin was not present in the uterine epithelium of oviparous S. equalis. Occludin was present in viviparous P. entrecasteauxii. Presence of occludin in viviparous species and not in oviparous species may indicate that tighter regulation of the paracellular pathway has arisen during the evolution of viviparity.

SEMINAL PLASMA REGULATES MMP-2, MMP-3 AND VEGF-C MRNA EXPRESSION IN THE PERI-IMPLANTATION MOUSE UTERUS

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Seminal plasma exposure elicits inflammatory changes within the uterine environment following insemination, with expression of pro-inflammatory cytokines and chemokines leading to the recruitment of macrophages and other leukocytes into the endometrium. The aim of this study was to examine the significance of these inflammatory changes in molecular regulation of endometrial receptivity for embryo implantation. We employed proteomics and real time RT-PCR techniques to evaluate the effect of seminal plasma on molecular mediators of tissue remodeling and early vasculogenesis at the time of implantation. Differential gel electrophoresis identified an increased abundance of a number of vasoactive serum proteins in addition to eight albumin variants in uterine tissues following exposure to seminal plasma. Real time quantitative RT-PCR analysis was used to
quantify molecular mediators of endothelial cell function or tissue remodeling in uterine tissues from estrous mice, or mice mated with intact, seminal vesicle deficient or vasectomised males. Messenger RNAs encoding matrix metalloproteinase (MMP)-2, MMP-3 and VEGF-C were significantly increased compared with estrous values in mice mated with intact or vasectomised males, but not seminal vesicle-deficient males. 1.3-fold, 1.5-fold and 1.3-fold increases respectively (all P < 0.05) were seen at the time of implantation due to prior seminal plasma exposure. These findings indicate that factors present in seminal plasma contribute to the molecular preparation of the uterus for pregnancy, providing an optimal environment for embryo implantation and subsequent placental development by facilitating increased vascular permeability and matrix remodeling at the time of implantation. Increased expression of MMPs and vasoactive cytokines in inflammatory leukocytes are likely to mediate these changes. These data begin to provide a molecular explanation for the adverse consequences for progeny of pregnancies initiated in the absence of seminal plasma exposure.

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LEPTIN IS CRITICAL FOR SUCCESSFUL IMPLANTATION IN MICE
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Leptin, a 16kDa polypeptide, has long been known to have a role in reproduction as exogenous leptin administration can reverse the sterility of ob/ob mice which lack functional leptin. Leptin binds to cell surface receptors (Ob-R) and signals via STAT/JAK signal transduction pathway. This ligand receptor system is mandatory for embryonic implantation in rodents as withdrawal of leptin infusion in ob/ob females shortly after fertilization impairs implantation. Leptin and its receptors are expressed in human and mouse endometrium. Leptin has been immunolocalized in human and mouse preimplantation embryos and promotes mouse preimplantation embryo development through Ob-R signaling. The aim of our experiment was to study the impact of blocking leptin signaling in the mouse endometrium by giving intrauterine injections of leptin antiserum on day 1 and day 3 of pregnancy. Mice were anesthetized and uterine horns were exposed using a dorsal midline approach and antibodies were injected (20µl) into the uterine lumen using a Hamilton syringe. Treated females (n=5) received anti-leptin antibodies (50 µg) while the controls (n=5) received similar concentrations of non-immune serum. After surgery, mice were placed in warm cages till recovery and euthanized on Day 10 of pregnancy. The uteri were extracted and the implantation sites were counted in each horn. Preliminary results show a significant reduction in the number of implanted embryos both on Day 1 (2.25 ± 1.25) and Day 3 (4.11 ± 1.08) of pregnancy in treated females as compared to control animals on Day 1 (13.55 ± 0.75) and Day3 (10.88 ± 0.9). These results confirm the previous findings that leptin signaling is critical for the implantation process.

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PRO-PROTEIN CONVERTASES: NOVEL REGULATORS OF ENDOMETRIAL PHYSIOLOGY AND IMPLANTATION
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Pro-protein convertases (PCs: furin, PC1, PC2, PACE4, PC4, PC6, PC7) are a family of serine proteases. They are responsible for post-translational processing and activation of inactive precursor proteins of growth factors, receptors and enzymes, thereby controlling their bioavailability. We recently demonstrated that PC6 is induced during and required for decidualisation both in the mouse and human (Nie et al. 2005, Okada et al. 2005). Whether other PCs have overlapping functions in the endometrium is not known. This study investigated the spatio-temporal expression of other PCs in the human endometrium. RT-PCR analysis demonstrated expression of furin, PACE4, PC4 and PC7 in human endometrium. In contrast, mRNA expression of PC1 and PC2 was negligible. Quantitative RT-PCR previously demonstrated significant up-regulation of PC6 expression with decidualisation of endometrial stromal cells in vitro (Okada et al. 2005). New data showed expression of furin, PACE4 and PC7 in the same cells, but expression levels did not correlate with decidualisation. Immunohistochemistry showed low intensities of stain for PACE4 and PC7 in endometrial stromal cells during menstrual cycle and decidualisation, with low to moderate intensities in endometrial glandular epithelia. In contrast, furin stained at moderate to high intensities in stromal cells during menstruation, proliferation, secretory phase and decidualisation. High furin levels were also immunodetected in endometrial glandular and luminal epithelia throughout the menstrual cycle and during early pregnancy. On the contrary, we previously demonstrated that PC6 protein is up-regulated in glands during the secretory phase, and is clearly induced during decidualisation of endometrial stromal cells (Nie et al. 2005). Thus, only PC6 seems to be closely associated with endometrial preparation for implantation. Furin, PC7 and PACE4 are likely to be involved in activation of regulators of a range of endometrial functions, such as menstrual tissue breakdown and repair, angiogenesis, growth and differentiation.


IDENTIFICATION OF PC6 SUBSTRATES INVOLVED IN HUMAN STROMAL CELL DECIDUALISATION USING PROTEOMICS

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Proprotein convertases (PCs) are a family of seven structurally related serine proteases which post-translationally process inactive precursor proteins into their biologically active forms. We have previously shown that PC6 (PC5) expression is induced during decidualisation of endometrial stromal cells both in the mouse and human. PC6 upregulation during decidualisation was further confirmed to be an absolute required for embryo implantation in the mouse.

In this study we utilised undifferentiated decidual cells derived from term decidua parietalis (closely resembling endometrial stromal cells) together with antisense morpholino oligonucleotides (MOs) to investigate the importance of PC6 in decidualisation in an _in vitro_ model. We observed a 50% decrease in decidualisation in cells which were pre-treated with PC6 MOs compared to treatment with a non-targeting control. These results strongly indicate that the action of PC6 either directly or via downstream substrate cleavage, is required for decidualisation.

To identify PC6 proteolytic substrates during decidualisation, we have performed 2D difference gel electrophoresis (2D DiGE) on lysates from cells treated with control and PC6 MOs. From this we have identified differences in protein profiles between the two groups which are likely to represent proteolytic targets of PC6 which may be required for decidualisation. We now hope to piece together these clues and hence further unravel the complexities behind this dynamic process essential for human embryo implantation.

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EFFECT OF POLYUNSATURATED FATTY ACIDS ON PGF 2α AND PGE 2 SYNTHESIS IN BOVINE ENDOMETRIUM AND TROPHOBLAST TISSUES.


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Omega-3 polyunsaturated fatty acids have the potential to improve fertility of dairy cows through the mediation of maternal prostaglandin synthesis at the time of pregnancy recognition (1). However, blastocysts also produce prostaglandins (2, 3) and any exogenous polyunsaturated fatty acids have the potential to alter both endometrial and embryonic prostaglandin synthesis. This study examined the effects of these acids on prostaglandin F2 alpha (PGF2α) and prostaglandin E2 (PGE2) production from endometrium and trophoblast tissues. Animals were slaughtered at either Day 16 or 17 of the estrous cycle. Uteri were flushed, and the embryo recovered (n=24). Endometrial tissues were collected and both these and trophoblast tissues were cultured in either ; media alone (DMEM) , or DMEM with 10uM docosahexaenoic acid (DHA), 10uM eicosapentaenoic acid (EPA), or 10uM linolaic acid (LIN). PGF2α and PGE2 in the culture media were analysed.

PGF2α and PGE2 production was greater from endometrium collected from pregnant compared to the non-pregnant endometrium (PGF2α: 14.6 ± 4.6 pg/mg vs. 3.8 ± 0.4 pg/mg w/w; PGE2; 78.3 ±16.2 pg/mg vs. 38.9 ± 4.7 pg/mg w/w for pregnant and non-pregnant animals). The addition of fatty acids only modified prostaglandin production in the pregnant endometrium. Of the concepti collected, 17 were 18.8 ± 0.7mm long and 7 were only 4.1 ± 0.9mm. Overall, the small concepti had a higher ratio of PGF2α to PGE2 compared to the large concepti when incubated in medium only (P< 0.05; 0.313 ± 0.103 vs. 0.662 ± 0.148 for the large and small concepti respectively). Addition of the fatty acids reduced this ratio in the small concepti but not the large ( P< 0.05).

In vitro prostaglandin production is higher in pregnant than non-pregnant endometrium. The effect of polyunsaturated fatty acids on concepti prostaglandin production is dependant on size. This indicates that when fatty acids are used to alter maternal prostaglandin synthesis the effects on concepti prostaglandin synthesis should be considered.

(1) Hwang et al., 1988 Prostaglandins 35:387-402
(2) Mattos et al., 2004 J Dairy Sci. 87:921-932
(3) Lewis et al., 1982 Biol Reprod. 2:431-439

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THE DISTRIBUTION OF OPIOID RECEPTORS IN THE HUMAN PLACENTA AND DECIDUA FROM EARLY PREGNANCY


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Background / Aims: The endogenous opioids, beta-endorphin, met-enkephalin and dynorphin are neurotransmitters that regulate mood and analgesia. They also modulate immune cell function and chemotaxis, and tumour cell proliferation, migration and invasion. Similar processes are crucial for maintaining uterine receptivity and regulating placentation in early pregnancy. Endogenous opioids are produced by the endometrium and placenta but, their role in uterine receptivity and
placentation, if any, remains unknown. The aim of this study was to explore if they may play a role, by identifying cellular targets of opioid action by localising mu, delta and kappa opioid receptors.

Materials and Methods: Placenta and decidua was collected from women undergoing elective termination of pregnancy at 7-10 weeks of gestation (n=6). Immunofluorescence staining for delta, mu and kappa opioid receptors was performed on OCT embedded tissue. Co-localisation of each receptor with CD56 or vimentin was carried out to identify uterine natural killer (uNK) and decidualized stromal cells, respectively. Western blotting was also carried out to detect the receptor proteins.

Results: Mu, delta and kappa receptors were localised to the syncytiotrophoblast, cytotrophoblast and trophoblast cell columns. The receptors were also distributed in the decidua but not the glandular epithelium. Dual staining showed that mu, delta and kappa receptors localised to stromal and uNK cells. Protein bands for delta (36 and 72kDa) and mu (48 kDa) opioid receptors were detected in placental and decidual tissue lysates following Western hybridization.

Conclusions: These findings suggest that endogenous opioids may be important and novel regulators of endometrial receptivity and placentation. They could regulate stromal cell decidualization, modulate their function and facilitate migration of peripheral NK cells into the uterus through chemotactic signalling. Placental growth and function and extra villous trophoblast proliferation and invasion may also be altered by endogenous opioids such as beta-endorphin, met-enkephalin and dynorphin.

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ESTROGEN STIMULATES MOUSE ENDOMETRIAL STEM-LIKE CELL PROLIFERATION

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Mouse endometrium (lining of the uterus) undergo cycles of growth and apoptosis with each estrus cycle. A well-known method to identify adult stem/progenitor cells and their location in the stem cell niche is the label retaining cell (LRC) approach. Epithelial LRCs have been identified in the luminal epithelium and stromal LRCs were found in perivascular regions beneath the luminal epithelium and near the endometrial-myometrial junction [1]. Since estrogen stimulates uterine epithelial proliferation in mouse endometrium during the estrus cycle, we hypothesized that estrogen would stimulate proliferation of epithelial and stromal LRCs, which would then drive endometrial growth. The overall aim of this study was to examine the effect of estrogen on the proliferation kinetics of mouse endometrial LRCs, before and after establishment of estrus cycles. The endometrium of postnatal (P3) mice was labeled for 3 days with bromodeoxyuridine (BrdU) followed by a chase period (CP) of 4 and 8 weeks. Mice were then ovariectomized and 1 week later an acute injection of 17-β estradiol was given and the uteri were harvested at 0, 2, 8, 16, 24 and 48 hours post estrogen treatment. Total LRCs (BrdU+), proliferating cells (Ki67+) and proliferating LRCs (BrdU+/Ki67+) were counted. Ki67 immunoreactivity was first observed at 8 hours post estrogen treatment in endometrial cells, almost exclusively in epithelial and stromal LRC (table).

All epithelial LRC proliferated within 8 hours, and were still Ki67+ at 48 hour, suggesting a second round of cell division. Only a small proportion of stromal LRC (~12%) initially proliferated within 8 hour (table) but it is uncertain if these cells underwent a second round of division or further LRCs were recruited into cell cycle. This study has demonstrated that estrogen induces both epithelial and stromal LRC mitosis in mouse endometrium, and suggests that epithelial stem-like cells may have an important role in driving endometrial growth and that only some stromal stem-like cells participate in this growth response.

A NOVEL ROLE FOR FRACTALKINE IN REGULATING HUMAN TROPHOBLAST EXTRACELLULAR MATRIX AND ADHESION MOLECULES.

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Embryo implantation requires attachment/adhesion of an activated blastocyst to a receptive endometrium, followed by precisely controlled trophoblast invasion; a deshesion molecules (AM) are key players. Chemokines regulate leukocyte chemotaxis via stimulation of AM. Fractalkine (FKN) exists as: (I) a membrane-anchored adhesion molecule that can capture/coordinate leukocyte migration in an integrin- and selectin-independent manner, (II) a soluble chemotactic peptide that cleaves from the cell surface. FKN is produced maximally by human endometrial epithelial and decidual cells around the time of implantation (1, 2). Both FKN and its receptor (CX3CR1) are detected in first trimester placenta and in trophoblast cells (3). Recombinant human FKN (rhFKN) stimulates human trophoblast migration (3) but the mechanisms underlying this are unknown. We hypothesized that FKN stimulates trophoblast migration by regulating extracellular matrix molecules (ECM) and AM expression in trophoblast cells. This study aimed to define the ECM and AM expressed by trophoblast cells and their regulation by FKN. Trophoblast cells (AC1M-88) used previously (3) were treated without and with rhFKN (10nM) for 18 hours. RNA was isolated and hybridized to ECM and AM oligo gene-arrays (n=3 arrays /treatment). Several of the most abundant genes identified in AC1M-88 cells, have previously been implicated in trophoblast invasion. FKN treatment altered mRNA expression of >30 genes compared to control. Several matrix metalloproteinases (MMPs) were up-regulated by FKN with MMP-12 showing a 10.9-fold increase. Intercellular adhesion molecule (ICAM)-1 and CD44 were increased 5-fold while integrin α6- and β5. E-cadherin and chondroitin sulfate proteoglycan 2 (CSPG2) genes were increased ≥ 2-fold. Osteopontin expression decreased 3-fold after FKN treatment. This is the first study to identify ECM and AM regulated by FKN in trophoblast cells suggesting mechanisms by which trophoblast cells migrate during implantation and early pregnancy.


INTERLEUKIN-11 PROMOTES MIGRATION BUT NOT PROLIFERATION OF HUMAN TROPHOBLAST CELLS

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Trophoblast growth and invasion of the uterine endometrium are critical events during human blastocyst implantation and subsequent placentation. Trophoblast cell proliferation, migration and invasion are tightly regulated by local factors produced within the trophoblast micro-environment. Interleukin-11 (IL-11) is essential for blastocyst implantation in mice¹,². In humans, IL-11 and its receptor IL-11 receptor alpha (IL-11Rα) are maximally expressed in decidual and in trophoblast cells during early pregnancy. While a role for IL-11 in decidualisation of human stromal cells is well established, its effect on trophoblast function is unknown. We hypothesised that decidual and/or trophoblast IL-11 has a role in regulating human extravillous trophoblast (EVT) proliferation and migration during implantation. We examined whether IL-11 and IL-11Rα are expressed by EVT cells in human first trimester implantation sites and whether IL-11 influences proliferation and migration of a human EVT-hybridoma cell-line used as a model for EVT. Cellular localisation of IL-11 and IL-11Rα in human first trimester tissues was assessed by immunohistochemistry. To confirm the biological responsiveness of EVT cells to IL-11, the effect of IL-11 on tyrosine phosphorylation (p) of signal transducer and activator of transcription (STAT) -3 was determined by Western Blot. Trophoblast proliferation was assessed by quantifying cell viability (Wst-1 assay) and DNA synthesis (bromodeoxyuridine (BrDU) assay), and migration was determined with an in vitro migration assay. Immunoreactive IL-11 and IL-11Rα were localised to interstitial EVT cells in vivo. IL-11 (1-100ng/mL): i) stimulated pSTAT3 but had no effect on STAT-3 abundance, ii) was without effect on trophoblast cell proliferation using either Wst-1 or BrDU assay, (iii) stimulated significant migration of trophoblast cells (100ng/mL) (p<0.01). These data demonstrate a role for IL-11 in human trophoblast migration but not proliferation during implantation, indicating an important role in placental development.

(3) Bilinski et al. (1998) Genes Develop. 12, 2234-2243
(4) Robb et al. (1998) Nat Med. 4, 303-308
ACCELERATED APOPTOSIS IS THE CAUSE OF GERM CELL LOSS IN GONADOTROPHIN-SUPPRESSED MEN
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Gonadotrophins play major roles in the regulation of germ cell development. Evidence have shown in rodents that gonadotrophic deprivation accelerates spermatogonial apoptosis1 and suggested that that this may be a cause of idiopathic infertility2. Increased apoptosis rather than reduced proliferation underlie the reduction of spermatogonial number in men with hormonally independent hypospermatogenesis3. However, data on the gonadotrophin regulation of spermatogonial proliferation are unknown. This study aimed to determine the relative contributions of changes in apoptotic and proliferation rates to the 70% reduction in germ cells number in men rendered gonadotrophin deficient by androgen/progestin contraceptive treatment1.

We used Bouin’s fixed, paraffin embedded tissues from normal fertile men that received testosterone enanthate (TE, 200mg im weekly) plus depot medroxyprogesterone acetate (DMPA, 300mg im once) for 2 or 6 weeks (n=5 per group) in order to maximally suppress gonadotrophins and spermatogenesis. Tissues from 5 normal fertile men were used as controls. Apoptosis and proliferation were identified by TUNEL (DNA fragmentation marker) and proliferating cell nuclear antigen (PCNA, cell cycle marker) labelling methods, respectively and assessed as a percentage of labelled over total cells by stereology in the following germ cell groupings; spermatogonia, primary spermatocytes and pachytene spermatocytes.

In response to T+DMPA treatment for 2 and 6 weeks, the percentages of TUNEL-labelled spermatogonia were increased to 438% (p=0.02) and 313 % (p=0.06) of control, respectively. There was also a trend for increased percentages of TUNEL positive primary and pachytene spermatocytes (385-308% and 109-332% control, respectively, p=0.15 & 0.23). No differences in the number of PCNA-labelled cells was observed for any germ cell type at either time point in T+DMPA-treated men compared to control.

This study demonstrates for the first time in men that the decline in spermatogonial (and possibly spermatocyte) number results from loss of gonadotrophins action as anti-apoptotic ‘survival’ factors, rather than from reduced proliferation. We are now identifying the pathways that underpin the apoptotic response.

(1) McLachlan et al 2002 J Clin Endocrinol Metab 87: 546
(2) Takagi et al 2001 Fertil Steril 76: 901

ANDROGEN RECEPTOR IS NOT ESSENTIAL FOR FEMALE REPRODUCTION BUT PLAYS IMPORTANT ROLES IN OPTIMISING FOLLICULAR DEVELOPMENT AND OVULATION
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The role(s) of genomic androgen action via the androgen receptor (AR) in female reproduction and follicle development remains unclear. Homozygous AR knockout (AR+/+) female mice were generated using Cre/LoxP recombination for in-frame excision of exon 3, encoding the second zinc finger essential for DNA-binding, to produce mutant AR Δ ex3 . Resulting ARΔ females were sub-fertile producing fewer pups/litter (ARΔ: 3.2 +/- 0.9; AR+/: 8.1 +/- 0.4, P<0.01), and delayed first litters (median time in days ARΔ = 61; AR+/: 22, P<0.05), yet all breeders were still fertile at 12 months. Gonadotrophin levels, somatic cell proliferation and follicle growth rates were not altered and follicle depletion was unaffected. This contrasts with another model of exon 1 ARΔ females with complete loss of AR function and expression, which have premature follicle depletion and are completely infertile at 40 weeks (1). Whether the extended fertility of AR Δ ex3 relative to the exon 1 deleted ARΔ (1) implies a role for ‘non-genomic’ AR actions in female fertility remains to be clarified. Atresia rates, however, were increased in antral follicles ( average atretic follicles/ovary ARΔ: 93.9 +/- 6.0; AR+/: 35.4 +/- 13.4, P<0.01) while reduced corpus luteum numbers at 10-12 (ARΔ 0 +/- 0; AR+/: 7.7 +/- 0.9, P<0.01) and 26 weeks (ARΔ 2.4 +/- 0.3; AR+/: 7.4 +/- 1.2, P<0.01) of age indicated reduced natural ovulation rates. Although reduced ovulation rates were overcome by gonadotrophins hyperstimulation (ARΔ: 25.2 +/- 4.1; AR+/: 27.4 +/- 2.5), fewer of the hyperstimulated oocytes ovulated reached metaphase II (ARΔ: 26.2 +/- 7.9; AR+/: 59.5 +/- 3.4, P<0.01). Therefore, although AR is not essential for female reproduction, disrupting genomic AR signalling in the ovary, leads to dysfunctional follicle development and reduced fertility; thereby demonstrating important roles for AR in optimising conditions for the developing follicle and ovulation.

SUCCESSFUL ARTIFICIAL INSEMINATION (AI) IN THE KOALA USING NEAT AND EXTENDED SEMEN COLLECTED BY ELECTROEJACULATION (EE)

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5Institute of Zoology, London, United Kingdom

Presently AI in the koala has been based on the insemination of fresh undiluted semen collected with an artificial vagina (1). While this approach has been extremely successful, further refinement and implementation of AI for use with cryopreserved semen will require protocols that incorporate diluted semen collected by EE. Recent studies have shown that koala semen is likely to have an "ovulation factor" such that over-dilution may result in ovulation failure (2). The current study determined whether AI of EEed neat and/or diluted semen was capable of inducing a luteal phase and/or resulted in the production of pouch young. All koalas were inseminated in the breeding season between day 2 and 5 of oestrus and subsequently monitored for evidence of parturition (day 35) and return of oestrus. Successful induction of a luteal phase was based on evidence of an elevated progesterone concentration 28 days after insemination (2). All semen samples were collected by EE and seminal characteristics recorded (3). The diluent used for semen extension was Tris-citrate glucose (TCG) which contained antibiotics but no egg yolk (4). AI was conducted on conscious koalas using a "Cook koala insemination catheter" and a glass rod used to mimic penile thrusting (1). Three insemination treatments were used; (A) 1mL of undiluted semen (n = 9); (B) 2mL of 1:1 diluted semen (n = 9); and (C) 1 mL of 1:1 diluted semen (n = 9). The results of the AI trial are shown in Table 1. This study has shown that it is possible to use both neat and diluted semen (1:1; 1 or 2 mL) to successfully produce koala offspring at conception rates similar to those achieved following natural mating. Interestingly, dilution of semen had no apparent detrimental effect on induction of a luteal phase following AI.

Table 1: Induction of a luteal phase or production of PY following AI in the koala using neat and diluted EE semen

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Induction of a luteal phase</th>
<th>Pouch Young</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7/9</td>
<td>4/9</td>
</tr>
<tr>
<td>B</td>
<td>7/9</td>
<td>4/9</td>
</tr>
<tr>
<td>C</td>
<td>6/9</td>
<td>4/9</td>
</tr>
</tbody>
</table>

(1) Int. Zoo Yearbook, 2003, 38: 160-172

MIFEPRISTONE ENHANCES ENDOMETRIAL REPAIR IN A MOUSE MODEL FOR BREAK-THROUGH BLEEDING ASSOCIATED WITH IMPLANON USE.

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Progestagen (P)-only contraceptives are safe and effective methods of long-term contraception. However, many users experience irregular and often prolonged episodes of uterine bleeding. A recent clinical trial showed that a low dose of mifepristone given to women using Implanon early during a bleeding episode reduced the number of bleeding days by approximately 50% compared to controls1. We have modified a mouse model of long term progestagen exposure2, and treated mice with mifepristone to elucidate the underlying mechanisms. Pseudopregnant mice received a decidual stimulus to one uterine horn and a subcutaneous etonogestrel implant on day 4 of pseudopregnancy. The other horn was left non-stimulated. As previously established1, complete endometrial decidualisation occurs 48 hours after the decidual stimulus is given, designated 0d. A single dose of mifepristone (200µg in arachis oil) was administered subcutaneously at 3d and mice were culled at 5d (n=5). Control mice received vehicle alone (n=5). Morphological changes were assessed following histochemical staining with particular attention to evidence of tissue breakdown and repair. Similar to the original model, the stimulated horn of vehicle-treated mice was highly decidualised at 0d and by 5d large blood vessels and substantial areas of tissue breakdown were evident. However, in mifepristone-treated mice endometrial repair was hastened, with areas of re-epithelialisation occurring at the interface of basal and decidual tissue, in some cases completely isolating the decidua from the regenerated tissue. In some instances the isolated decidua was expelled from the uterine lumen as distinct fragments. These processes were not observed in control tissues. The non-stimulated horn did not undergo breakdown or repair.
In summary, this model shows some similar endometrial characteristics to women using P-only contraceptives and provides a unique opportunity for functional studies. We show that the reduction in bleeding days experienced by women receiving mifepristone in a recent clinical trial is likely attributed to enhanced endometrial repair.


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OSTEOBLAST SPECIFIC FACTOR -2 AND OSTEOPONTIN ARE PRESENT IN ENDOMETRIOTIC TISSUES.
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Endometriosis is a common disease in reproductive aged women wherein endometrial tissue grows outside the uterine cavity, leading to chronic pelvic pain, painful periods and subfertility. Current treatments for this debilitating disease are not curative and have significant side effects such as bone loss. A microarray analysis of lesions from the nude mouse model of endometriosis has shown that osteopontin (SPP-1) and osteoblast specific factor -2 (OSF-2) transcripts are highly elevated in these endometriosis-like tissues. It is known that in osteoblasts both these factors are up regulated by the RUNX-2 (CBFa-1,) transcription factor, which has also been demonstrated by immunohistochemistry in the nude mouse lesions. It was our aim to identify SPP-1 and OSF-2 in human endometriotic tissue to assess the possibility that members of the RUNX-2 bone-remodeling pathway could be therapeutic targets for treating endometriotic disease. Eutopic and ectopic endometrial samples were collected from naturally cycling women aged between 18 and 45. Immunohistochemistry using anti-osteopontin antibodies was carried out on paraffin sections derived from 4 patient samples and the levels of OSF-2 mRNA were quantified using RT-PCR techniques in a separate cohort of 5 endometriotic samples. The presence of osteopontin was demonstrated in 4/4 paired endometriotic and eutopic samples with staining in the later being more scarce and predominantly perivascular. OSF-2 mRNA was present in endometriotic tissue from 5/5 patients. These results demonstrate for the first time the presence of osteopontin and OSF-2 in endometriotic tissue. If the RUNX-2 bone remodeling pathway is dysregulated in endometriotic tissue, its manipulation could modify or treat endometriotic disease. Further experiments will clarify the expression and role of the RUNX-2 associated proteins in endometriotic disease.

ENDOMETRIAL INTERLEUKIN 11 IS DYSREGULATED IN INFERTILITY DURING THE IMPLANTATION WINDOW.
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The human endometrium provides a hostile environment to embryo implantation except during a limited phase of the menstrual cycle known as the “window of receptivity”. Endometrial IL-11 and IL-11 receptor (R) \(\alpha\) and LIF are dysregulated in some women with endometriosis. We hypothesized that women with infertility of unknown etiology have dysregulated endometrial IL-11, IL-11R\(\alpha\) and/or LIF. We aimed to compare mRNA expression and immunoreactive IL-11, IL-11R\(\alpha\) and LIF in infertile and fertile women during the receptive window. Immunohistochemistry and quantitative-real-time-RT-PCR for IL-11, IL-11R\(\alpha\) and LIF were performed on endometrial tissues from women with at least 2 years of primary infertility (\(N=13\)) and fertile women (\(N=11\)) between days LH+4 and +10. IL-11 immunostaining in glandular epithelium (GE) was significantly lower in a cohort of infertile compared to fertile women (\(P<0.05\)). Moderate to low staining for IL-11 and IL-11R\(\alpha\) was seen in luminal epithelial (LE) and vascular endothelial cells (EC), and low in stromal and vascular smooth muscle cells (SMC). LIF immunoreactivity in GE was absent in a sub-population of infertile women. By contrast, GE LIF immunostaining was found in all tissues from fertile women. In the tissues that exhibited LIF staining, the intensity in GE, LE, SMC and EC was moderate to low and not different between infertile and fertile women. Decidualized stromal cell (D), IL-11 and IL-11R\(\alpha\) intensity was moderate to high in fertile women and low in infertile tissues. By contrast, LIF staining was minimal in D in infertile and fertile women. Endometrial IL-11, IL-11R\(\alpha\) and LIF mRNA expression were not significantly different between infertile and fertile women suggesting the dysregulation may be at the level of translation or processing rather than transcription. These data provide important new information suggesting IL-11 is dysregulated in sub-populations of infertile women and support a role in preparation of a receptive endometrium.

INTERLEUKIN-1B -511 POLYMORPHISM IS ASSOCIATED WITH PREECLAMPSIA

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Pregnancy complications including recurrent miscarriage, preeclampsia (PE), intrauterine growth restriction (IUGR) and preterm birth affect more than 20% of all pregnancies each year. The interleukin-1b (IL-1b) gene contains several single nucleotide polymorphisms (SNPs), which encode increased cytokine expression. These have been implicated in several pregnancy complications. We determined the prevalence of two SNPs (-511 and +3953) in the IL-1b gene within members of the general South Australian community and in mothers, fathers and babies at risk of a pregnancy complication. Blood was collected from adult volunteers (n=142) and from women attending the WCH High Risk Pregnancy clinic (n=69), their partners (n=45) and cord blood or tissue was collected from neonates (n=37). Genotype was ascertained by RFLP-PCR and additional clinical information collected. Women were followed through pregnancy and classified into control or patient groups (recurrent miscarriage n=21, PE n=14, IUGR n=10, preterm birth n=3) after delivery. Preliminary data suggest the T/T genotype in the IL-1b -511 SNP is twice as common in PE trios and babies when compared to normal pregnant controls (p=0.031). As expected maternal age is increased and birthweight decreased in PE pregnancies compared to controls. There have been no significant findings with the IL-1b -511 SNP in the other patient groups or with the IL-1b +3953 SNP.

Table 1: Frequency of IL-1b SNPs in the general South Australian community

<table>
<thead>
<tr>
<th>Genotype</th>
<th>C/C (%)</th>
<th>C/T (%)</th>
<th>T/T (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1b -511</td>
<td>39</td>
<td>46</td>
<td>15</td>
</tr>
<tr>
<td>IL-1b +3953</td>
<td>67</td>
<td>26</td>
<td>2</td>
</tr>
</tbody>
</table>

For the first time the frequencies of the IL-1b -511 and +3953 SNPs have been determined within the SA community. Further recruitment and genotype analyses of patient groups will increase the power of this study in time. At present there appears to be an association between a T/T genotype at the IL-1b -511 SNP and PE. However, a larger number of samples is needed to validate this finding.

THE GENE ENCODING THE CONSTANT REGION OF THE HEAVY CHAIN OF IMMUNOGLOBULIN G IS DIFFERENTIALLY EXPRESSED IN HUMAN DECIDUA IN ASSOCIATION WITH PREECLAMPSIA

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Human pregnancy is a complex and precisely choreographed process; from conception and implantation of the blastocyst, formation of the placenta, maternal adaptation to pregnancy, through fetal development, and finally to labour and birth. One of the crucial elements in a successful pregnancy is the accommodation by the mother of her genetically distinct offspring. It is the intimate and complex interaction between the fetal cells of the placenta and the maternal cells that line the pregnant uterus that has generated considerable interest. It is in the milieu of the maternal/fetal interface of the uterus that two genetically distinct cells must accommodate each other for their mutual benefit. An inability to do so may contribute to a number of complications of pregnancy including preeclampsia.

Biochemical and epidemiological studies suggest that the immune system plays an important role in preeclampsia. An aberrant interaction at the maternal/fetal interface between the genetically distinct fetal trophoblast cells and the cells of the maternal decidua has been proposed as an initiating factor in preeclampsia. Thus, the aim of this study was to determine the decidual gene expression status in preeclampsia of one of the key components of the adaptive immune system. Total RNA was extracted from decidua collected from women with normal pregnancies (n=12) and those complicated by preeclampsia (n=12). Reverse Northern analysis was performed on 72 cDNAs from human decidua and differentially expressed genes identified were analysed further using semi-quantitative RT-PCR and Northern blot analysis. Expression of the gene encoding the constant region of the heavy chain of immunoglobulin G was shown to be down-regulated in association with preeclampsia.

These data support the hypothesis that immune maladaptation may play an important role in the pathogenesis of preeclampsia.
THE ROLE OF THE NOVEL SPERM PROTEIN SPRASA IN INFERTILITY

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Introduction: SPRASA, a recently discovered acrosomal protein, is the target of antisperm antibodies from some infertile men. Antibodies reactive with SPRASA can inhibit fertilisation [1, 2]. SPRASA is related to the α-lactalbumin/c-type lysozyme family of proteins and appears to be expressed only in the testis/sperm but its function is not yet known. The aim of this work is to identify the role of SPRASA in fertility/infertility.

Methods: Computer-based analysis of publicly available databases was used to identify SPRASA orthologues. DNA was extracted from peripheral blood from 72 fertile and 26 infertile couples. The SPRASA gene sequences from these individuals were determined by automated sequence analysis.

Results: Database searches revealed SPRASA orthologues are limited to mammals including mouse, rat, dog, chimpanzee and bull but that human SPRASA contains an additional exon and transcription start site (ATG) which is absent from the non-primate orthologues. Sequence analysis of the SPRASA orthologue genes has identified a highly conserved 10 amino acid motif that appears to distinguish SPRASA from related proteins of the c-type lysozyme family. A phylogenetic tree has been constructed showing the bovine orthologue is the least conserved, compared to human. DNA sequence analysis of the SPRASA gene from infertile and fertile couples has identified a TGC base triplet located immediately 5’ to the first transcription start site that is repeated four times in most individuals. However, two infertile males are homozygous for the insertion of a fifth TGC repeat.

Discussion: SPRASA has been highly conserved during mammalian evolution suggesting it has an important function in reproduction. Human SPRASA has an N-terminal extension that makes it a transmembrane protein. The importance of the homozygous TGC repeat variant is unclear but this could potentially alter levels of SPRASA expression in affected individuals.

References:

THE INFLUENCE OF REGULATED NUCLEAR TRANSPORT ON PRIMORDIAL GERM CELL LINEAGE SPECIFICATION

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Novel regulatory mechanisms driving cell fate choice have recently been revealed, whereby access to the nucleus is regulated through production and localization of specific nuclear transport factors. We have modeled developmental switches in early embryogenesis using murine embryonic stem cell (ES) differentiation technologies to specifically address this phenomenon. We have determined by RT/PCR that the nuclear transport factors importins α1-4 and β1, β1-2, and Ranbp5 are all expressed in undifferentiated ES cells. Using immunofluorescence and quantitative analysis of confocal microscopy, the intracellular distribution of several of these proteins was measured. While the nucleo-cytoplasmic ratio of the importins α1 and β1, and Ranbp5, remains consistent in all cells within a colony, the α1 and β1 ratios appear to vary between cells. We hypothesize that such variation indicates a differential need for the specific cargoes of these transport factors within distinct subcellular compartments, and may correlate with cell cycle or differentiation status. We next differentiated ES cells as embryoid bodies (EBs) and documented the emergence of multiple lineages, including primordial germ cells (PGCs) through RT/PCR analysis of lineage-specific markers. There is marked alteration in the subcellular distribution of nuclear transport proteins as differentiation progresses. In addition, the nucleo-cytoplasmic ratio of these proteins varies across the EB, potentially correlating with the distinct needs of different lineages. To investigate lineage-specific nuclear transport needs, we have developed directed differentiation methodologies whereby, through the action of the TGFβ1 ligand, BMP4, we are able to enhance and maintain a population of cells within an EB that express PGC markers. We are now analyzing the effect of functional alterations in specific nuclear transport factors on the specification of this lineage. This study represents a key paradigm shift in our understanding of the underlying mechanisms that drive embryonic, and particularly reproductive, development.
PRIMORDIAL GERM CELL SPECIFICATION: AN ALTERNATIVE MAMMALIAN MODEL.
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Despite the importance of primordial germ cells as the precursors of the male and female gametes, there have been relatively few studies of marsupial germ cells. In contrast to the mouse, marsupial embryos develop in a planar manner until the early head fold stage. We have used the tammar wallaby *Macropus eugenii* to investigate marsupial germ cell specification. Several antibodies that recognise murine germ cells (vasa, SSEA-1, EMA-1, stella, fragilis and Oct4) were tested on tissues of the developing tammar. Both EMA-1 and SSEA-1 recognised tammar germ cells as well as some somatic cells in all three germ layers in the posterior region of the primitive streak from the 2-3 somite stage. EMA-1 became relatively germ cell-specific at the early fetal stage, SSEA-1 at the late fetal stage. The vasa antibody stained both germ and somatic cells throughout pre-natal development whereas the Oct4 antibody that we used stained only somatic cells at these stages. To investigate germ cell specification using reciprocal tissue transplants, we are optimising culture conditions for peri-gastrulation tammar embryos. Embryos were cultured at 37°C in Dulbecco's Modified Eagle's Medium (DMEM) using static culture with either wallaby serum or fetal calf serum and 6% CO2 in air or 5% CO2/95% oxygen. There were no differences between the two serum types, but embryos cultured in the higher oxygen concentration developed further and maintained near normal morphology compared to those cultured in air. We were able to maintain development of embryos in these cultures for up to 3 days, though some abnormalities were evident after 30 hours. Post-nodal stage embryos developed beating heart tubes and up to 20 somites *in vitro*; in contrast pre-nodal embryos developed poorly. Culture of peri-gastrulation tammar embryos therefore has the potential to provide a model for the experimental study of marsupial germ cell specification.

ESTABLISHMENT OF MOLECULAR MARKERS FOR BOVINE TESTIS TRANSPLANTATION
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Knowledge of the molecular mechanisms underpinning spermatogenesis is far less developed in bovine and other farm animals than for rodents. The technique of testicular germ cell transplantation has recently become well established for studies of spermatogenic stem cells (SSCs) in rodents, but attempts to transplant domestic animal germ cells into the mouse or autologous hosts are rarely successful. Crucial to progress in this area is identification of markers that will unequivocally distinguish donor and recipient somatic and germ cells after transplantation. We hypothesized that bovine SSCs would share molecular markers common to rodents, and in this study we screened key markers identified previously in the mouse. Primers corresponding to β-actin (a control), VASA, GFRα1, stem cell factor (SCF), and glial cell-derived neurotrophic factor (GDNF) were designed from a public access bovine genome database to amplify 200-400bp regions of these genes for subsequent use *in situ* hybridisation. RT/PCR was performed on RNA from bovine testes from 10 g to >100g, collected from prenatal to adult animals, and from fetal bovine testis and cell preparations from immature testes. All primer pairs amplified products of the predicted size which were subsequently subcloned and verified by sequencing. Our *in situ* hybridization experiments to date, using a digoxigenin-labelled cRNA probe verified Northern blot, identify VASA mRNA in germ cells of immature and adult testes. The strongest VASA signal was in pachytenne spermatocytes. In relationship to our objective of producing markers that distinguish host and donor cells, our PCR amplification of SCF, GDNF and β-actin amplify targets from bovine but not from mouse. These tools will be appropriate for determining whether murine SSC markers are common to bovine SSCs and developing male germ cells, and they can now be applied to the analysis of bovine germ cell transplantation and grafting experiments.

MAGNETIC ACTIVATED CELL SORTING FOR PURIFICATION OF BOVINE TYPE A SPERMATOGONIA
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Male germ cell transplantation involves two steps: isolation of germline stem cells and preparation of the recipient. Purification of stem cells might improve the efficiency of transplantation. Magnetic Activated Cell Sorting (MACS) has been employed as a fast and effective way to purify cells from mixed populations; however, lack of germ cell specific markers could limit the technique for testicular germ cell transplantation.
Here we screened three lectins *Dolichos Biflorus Agglutinin* – fluorescein isothiocyanate (DBA-FITC), *Dolichos Biflorus Agglutinin* - tetramethyl rhodaminyl-isothiocyanate (DBA-TRITC) and *Datura Stramonium Agglutinin* - fluorescein isothiocyanate (DSA-FITC) for staining testicular cells. DBA-FITC specifically labeled gonocytes and type A spermatagonia from immature and pubertal testes while DBA-TRITC and DSA-FITC stained all testicular cells. DBA binding to spermatogonia did not differ between freshly isolated cells (30 min after trypsin treatment) and cells stored at 4°C overnight after isolation. Two preparations of testicular cells containing different percentages of type A spermatogonia were labeled with increasing doses of DBA and examined by flow cytometry to determine the optimal concentration of DBA-FITC for staining testicular germ cells. Mean fluorescent intensity and percentage of positive cells plateaued at 10μl of DBA-FITC (2mg/ml) when labeling 10⁶ cells in a 100μl volume. Three methods for purifying DBA-FITC labelled cells by MACS were examined. Microbeads coated with antibody to FITC were examined at two doses with a single column passage, and the lower dose of microbeads was examined with two column passages. All methods resulted in a significant increase in DBA positive cells from around 12% of input cells to 45-60%. A single column passage with the higher dose of microbeads produced the best purity (55.61 ± 3.33% DBA-FITC positive cells) and recovery rate (4.59 ± 0.38 % of input cells). In summary, MACS can provide highly enriched type A spermatogonia for testis germ cell transplantation and therefore could significantly increase the success of this technique.

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**ACTIVIN EFFECTS GERM CELL NUMBER AT SPECIFIC DEVELOPMENTAL AGES IN THE FETAL MOUSE TESTIS**

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The Transforming Growth Factor-β (TGFβ) superfamily of ligands are known to influence male germ cell development and testis function. In our ongoing investigations of genetically modified mice with different bioactive levels of activin, we noted that testicular germ cell numbers at birth are increased in the absence of activin (Loveland et al 2005) and have identified downstream target genes via SuperArray analyses: Inhibin alpha, Smad 5, Insulin-like growth factor 1 (IGF-1), p15INK4b, ALK-6 and TIMP-1. To elucidate the timing of this germ cell increase we looked at the fetal testis. In this study we compare gonad volume, primordial germ cell (PGC) number and Sertoli cell number in mice at 13.5 and 15.5 days post coitum (dpc) and examine with 0, 1 or 2 copies of the activin β A subunit gene, corresponding to the genotypes activin βA null, heterozygous and wildtype genotypes respectively. Bouins fixed methacrylate embedded testes sectioned at 25 μm were used to determine testis volume by the fracionator method; activin βA levels had no effect on fetal testis volume at 13.5 and 15.5dpc. Application of stereological quantitation using the optical dissector revealed that activin βA levels had no effect on Sertoli cell number at both fetal age studied. In contrast, at 15.5dpc the lack of activin βA caused a significant increase in PGC number in knockout compared with wildtype testes (p<0.05), while there was no change in PGC number between the three genotypes at 13.5dpc. This finding is in agreement with studies that show activin as a growth suppressor for germ cells in the testis. Our ongoing analyses of these fetal testes with markers of proliferation (BrdU and PCNA) and apoptosis (TUNEL) should reveal the exact mechanism for this increase in PGC number at the fetal ages studied.


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**REGULATION OF C-KIT RECEPTOR IN GERM CELLS OF THE RODENT TESTIS BY MEMBERS OF TGF-BETA SUPERFAMILY**

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The Stem cell factor (SCF)/c-Kit ligand receptor expression and signalling in the testes is vital for normal male reproductive function with c-Kit as a marker of differentiating spermatogonia. While Sertoli cell production of SCF is enhanced by FSH, regulation of c-Kit in spermatogonia is unknown. We investigated the influence of activin A in the expression of c-Kit in germ cells passing through the first wave of spermatogenesis, since activin A is high at this time point and is known to down regulate c-Kit mRNA and protein expression during erythroid maturation (Hino et al, 1995). This hypothesis was first investigated in a transgenic mouse model using Sertoli-germ cell co-culture. The *Inhba* mouse in which the *Inhbb* coding sequence replaces the *Inhba* locus resulting in lower levels of bioactive activin. Quantitative PCR measurement of c-Kit mRNA in day 7 testes of *Inhba* showed a significant increase (p<0.05) in c-Kit expression in *Inhba* animals as compared to the *Inhba* and *Inhba* mice. SCF measurements indicated that there was significant suppression of this mRNA (p<0.05) in *Inhba* and *Inhba* mice. In the co-cultures of Sertoli-germ cells from day 8 testes, treatment for 24 hours with human recombinant activin A significantly reduces (p<0.05) c-Kit mRNA. Quantitative expression of germ cell marker VASA ruled out germ cell apoptosis in activin treated samples as a possible mechanism of c-Kit mRNA suppression. These findings provide the first *in vivo* identification of an endogenous regulatory factor for c-Kit at the onset of spermatogenesis.
EXOGENOUS GROWTH DIFFERENTIATION FACTOR 9 DURING IN VITRO MATURATION OF OOCYTES IMPROVES SUBSEQUENT EMBRYONIC DEVELOPMENT AND FETAL OUTCOME

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The viability of an embryo is dependent on the developmental competence of the oocyte it is derived from. Recently, the existence and necessity of a bi-directional regulatory loop between oocytes and their somatic cells have become evident. The aim of this study was to assess the effects of the oocyte paracrine factor growth differentiation factor 9 (GDF9) addition during mouse oocyte in vitro maturation (IVM), on subsequent embryo and fetal development.

COCs were aspirated from antral follicles of pre-pubertal (CBA/B6F1) mice at 46h post eCG and matured at 37 °C in 6%CO₂:5%O₂ for 17h in Waymouth's medium + 5% FCS with or without 50mIU/ml FSH and 10ng/ml EGF, recombinant mouse GDF9 (200ng/ml) or the equivalent v/v control parent cell line 293H conditioned medium. Matured oocytes (n=1106) were fertilised and cultured to the blastocyst stage in G1.2/G2.2 media at 37 °C in 6%CO₂:5%O₂. Blastocysts were pooled and either transferred to pseudo-pregnant Swiss females or differentially stained. Pregnancy outcome was assessed on Day 15 of pregnancy.

With FSH/EGF, GDF9 increased cumulus expansion (3.1±0.1 cumulus expansion index vs. 2.4±0.1;P<0.05). Although there was no significant effect of GDF9 on fertilisation, rate of development or blastocyst percentages, (83% vs. 75%), GDF9 significantly increased blastocyst total cell number (P<0.01), with greater differences noticed in inner cell mass (P<0.01) than trophectoderm cell numbers (P<0.05). Accordingly, implantation was not affected (83% vs. 77%), but fetal development was almost doubled with GDF9 addition (39% vs. 21%;P<0.05). In the absence of FSH/EGF, GDF9 had no effect on outcomes measured.

This study demonstrated that with FSH/EGF, exogenous GDF9 addition during IVM improved blastocyst quality and subsequent fetal viability. These findings highlight the importance of appropriate oocyte-somatic cell interactions and have significant implications for the development of IVM culture media as impaired developmental competence of IVM oocytes may partly result from GDF9 deficiency.

FERTILITY STUDY OF COMPLEMENT-3 IN MICE

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Human oviductal cells produce complement-3 (C3) and its embryotrophic derivative, iC3b in the presence of embryos. We proposed that C3-deficiency would lead to fertility impairment in mice in vivo. To determine the physiological significance of this protein in reproduction, heterozygous mice carrying the mutated C3 gene (C57BL/6J-C3tmCrr) were purchased from Jackson Laboratory. Crossing of these mice (C3+/−) generated mice carrying the homozygous (C3−/−) and mutated C3 (C3+/−) genes. C3−/− and the wild type C3+/+ were allowed to cage with males of the same genotype for 6 months and their fertility was examined. Both genotypes are fertile and produce viable pups. The number of litters per week born from C3−/− pairs (0.116±0.05) were significantly smaller than those in C3+/+ pairs (0.168±0.04). There were no significant difference between the mean numbers of pups per litter, mean born weight and mean litter size at wean between the two groups. However, the mean pup weight at weaning of C3−/− (8.1±1.2 g ) was significantly smaller than that of C3+/+ pairs (8.6±1.3 g). Although C3 protein could not be detected in the C3−/− mice serum by Western blot, C3 immunoreactivity and mRNA was detected in the oviduct and liver tissues homogenate, suggesting the presence of mutated C3 molecules in these animals. These mating results suggested that the C3−/− mice require longer getting pregnant and the resulting pups are smaller in size at weaning. The biological activity of the mutated C3 molecule on embryo development remains to be investigated.

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VARIABLE EXPRESSIVITY OF THE TUMOUR SUPPRESSOR PROTEIN P53 IN HUMAN EMBRYOS
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Assisted reproductive technology is associated with a rate of embryo wastage. There are likely to be many contributing factors to this, including the age of the parents, aetiology of infertility, and the duration and methods of embryo culture in vitro. Embryos in culture are likely to be subjected to multiple stressors, and a number of lines of evidence indicate that these may reduce the developmental potential of embryos. In a mouse model culture stressors lead to increased expression of p53, while ablation or inhibition of p53 improved embryo viability. This study investigates the status of p53 expression with human embryos produced by IVF.

Excess human ART embryos were donated for research with informed consent under NHMRC licence 309702B. Gametes were fertilised in Sydney IVF Fertilisation media, and then cultured in Sydney IVF Cleavage media (48h) followed by cultured in Sydney IVF Blastocyst media (William A. Cook, Aust). Embryos were then frozen using a Sydney IVF Blastocyst Freezing Kit (Cook). Upon thawing, embryos were immediately fixed in formaldehyde and subjected to immunolabelling for P53. Expression of p53 was visualized by confocal microscopy.

Negative controls were incubated with isotype control immunoglobulin and showed negligible immunolabelling. All embryos showed some p53 expression, although expression was heterogenous within and between embryos. In general there was an a negative association between the apparent developmental normality and morphology of embryos and the extent of p53 expression – embryos that were of advanced stage and normal morphology showed lower levels of p53 expression and little nuclear accumulation, while there was high levels of cellular and nuclear expression of p53 in retarded or degenerating embryos.

These results infer that human embryos produced in vitro respond in varying degrees by the expression of p53. It remains to be determined wether the variability of expression in humans is causative of the retarded or degenerate morphology observed.

DEMONSTRATION OF AN ASSOCIATION BETWEEN THE EXTENT OF CULTURE STRESS AND THE EXPRESSION OF P53 IN MOUSE EMBRYOS.
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Embryo culture techniques underpin technologies used in assisted reproduction, embryonic stem cell derivation, and somatic cell nuclear transfer. Embryo culture commonly reduces the developmental potential of embryos. In the mouse model, reduction of developmental potential relates to mouse strain and the developmental stage at which culture is initiated (and duration of culture). Thus, C57BL6 (inbred) is more susceptible than hybrid F1B6CBA, and culture from the zygote stage (96 hour culture) reduces potential more than from the 2-cell stage (72 hour culture). Recent evidence indicates P53 expression is normally latent within preimplantation embryos, but culture can breach this latency in susceptible embryos. This study was undertaken to determine whether any association exists between these factors influencing susceptibility of embryos to culture stress and the expression of P53 in resulting blastocysts.

We compared the expression of P53 mRNA (QPCR) and protein (confocal immunoflourescence) in blastocysts collected: fresh from the reproductive tract 3.5 d.p.c.; as zygotes cultured for 96 hours; or as 2-cell embryos cultured for 72 hours. Embryos were collected from C57BL6 or F1B6CBA females mated with isogenic males, and cultured in modHTF media with BSA (3 mg/ml). An association between P53 expression (and nuclear localization) and culture stress was observed. Thus, embryos cultured from the zygote stage had markedly more expression than embryos cultured from the 2-cell stage. Cultured C57BL6 embryos gave higher levels of expression than cultured F1B6CBA embryos. Surprisingly, we found no evidence of accumulation of S15-phosphorylated P53, implying the ATM/ATR pathway may not contribute to this expression.

These results confirm that there is an association between the expression of p53 and the extent of loss of viability that occurs with the culture of mouse embryos in vitro. It shows for the first time that p53 expression was not apparently associated with phosphorylation by ATM/ATR.
P53 EXPRESSION IN MOUSE AND HUMAN SPERM

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Mammalian spermatozoa lose fertilising capacity rapidly after their addition to culture media, and seem to be susceptible to a range of genotoxic and non-genotoxic stressors in vitro. The regulation of such responses is yet to be fully defined. P53 is an important regulator of responses to external stresses by many cell types. The status of p53 in mammalian spermatozoa has not been extensively investigated.

This study examined the expression of p53 in mouse and human sperm and investigated the effect of genetic deletion of p53 from mouse sperm on their fertilising capacity. P53 protein was detected in sperm by both western analysis and immunofluorescence. It was present in both mouse and human sperm. It was not detected at high levels in all sperm, rather heterogeneity in the levels of expression was characteristic. When detected, p53 was commonly within the midpiece region of the sperm. Sperm collected from p53+/− males were used in in vitro fertilisation to assess the relative fertility of wildtype compared to p53 null sperm. It was expected that p53+/+ and p53+/− embryos would result in equal proportions. Embryos were collected 24h after in vitro fertilisation and subjected to genotyping by PCR. There was a significant skew in favour of p53+/+ embryos compared to p53+/− embryos.

The results show that there is heterogenous expression of p53 in the midpiece of mouse and humans sperm. P53 is reported to be a transcription factor, but also influences mitochondrial function. Its location within sperm infers that any potential actions are non-nuclear. The apparent reduction in fertility of p53 null sperm in vitro infers that p53 function may be important for the fertility of the mouse sperm in vitro.

COMPARISON OF A SINGLE MEDIUM WITH SEQUENTIAL MEDIA FOR THE CULTURE OF SIBLING HUMAN EMBRYOS TO THE BLASTOCYST STAGE

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3LifeGlobal LLC, Guelph, Ontario, Canada

Introduction: It has been suggested that sequential culture media are required to meet the changing requirements of the developing embryo. Conversely, it has been shown that a single medium can support the development of the human embryo to the blastocyst stage. The purpose of this study was to use sibling human zygotes to directly compare a single medium with sequential media for development to the blastocyst stage.

Methods: Oocytes were retrieved from 42 women, 20-38 years of age (29.7 ± 5.9, mean ± S.D). The morning after fertilisation (Day 1), the zygotes were randomly divided and cultured individually in 15 µl droplets of Global medium (IVFonline, N = 268) or Early Cleavage Medium (ECM, Irvine Scientific, N = 283). On Day 3, the embryos in Global medium were transferred to fresh droplets of Global medium; those in ECM were transferred to droplets of Multiblast Medium (Irvine Scientific). All media were supplemented with 10% SSS (Irvine Scientific). On Day 5, the best one or two embryos from both culture treatments were selected for transfer. Proportions were compared by Chi-square analyses; all other measures by Kruskal-Wallis tests.

Results: The results of evaluation of the embryos on Days 3 and 5 are shown in the table below. There were no significant differences between the culture treatments for cell numbers or fragmentation on Day 3, but compaction was significantly greater in Global medium. The ICM and trophectoderm scores on Day 5 were not different between media treatments. Development to blastocyst, development to expanded blastocyst, and selection for transfer were significantly greater for embryos cultured in Global medium than in Irvine media.

Conclusion: The results indicate that a single medium (Global) is at least as good as sequential media (Irvine ECM and Multiblast) for the culture of human embryos to the blastocyst stage.
SIGNIFICANCE OF EARLY DEVELOPMENTAL MARKERS IN HUMAN CRYOPRESERVED EMBRYOS
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Introduction: In human IVF, early syngamy and cleavage are associated with increased implantation potential. In post-thaw cryopreserved embryos, extent of blastomere survival and subsequent overnight cleavage are associated with implantation potential. In this study, we have investigated the relationship between early developmental events and outcomes following cryopreservation.

Methods: A retrospective analysis of 3370 frozen/thawed day 2 embryos was conducted to establish the impact of early developmental markers on post-thaw outcomes. All embryos had been assessed at 23/24 hours post insemination for syngamy status and categorised as 1) early cleavage (EC), 2) completion of nuclear envelope breakdown (NEBD) or 3) both pronuclei visible (2PN). Post-thaw survival was defined as at least 50% of blastomeres surviving.

Results: A statistically significant difference was found between post-thaw survival of 4-cell (1791/2153; 83%) and non-4-cell (784/1212; 64.7%) embryos (p < 0.01, χ2). We, therefore, compared survival and subsequent cleavage in relation to syngamy status in 4-cell embryos only (Table I).

Table I: Cryopreserved 4-cell embryos: Survival and cleavage rates versus syngamy status at 23/24 hpi

<table>
<thead>
<tr>
<th>23/24 hpi status</th>
<th>Total embryos thawed</th>
<th>Survival</th>
<th>Cleavage</th>
</tr>
</thead>
<tbody>
<tr>
<td>2PN</td>
<td>1005</td>
<td>852 (85%)</td>
<td>601 (70%)</td>
</tr>
<tr>
<td>NEBD</td>
<td>785</td>
<td>652 (83%)</td>
<td>561 (86%)</td>
</tr>
<tr>
<td>EC</td>
<td>368</td>
<td>287 (78%)</td>
<td>264 (92%)</td>
</tr>
</tbody>
</table>

Survival of thawed 4-cell embryos was not significantly affected by syngamy status at 23/24 hpi, whereas the proportion of surviving 4-cell embryos that resumed mitosis was significantly (p < 0.001, χ2) higher in the NEBD (561/652; 86%) and EC (264/287; 92%) groups than in the 2PN group (601/852; 70%).

The implantation rate of thawed embryos which had entered syngamy by 23/24 hpi i.e the NEBD+EC groups (18.3%; 191FHs /1046 embryos transferred ) was significantly (p<0.01, χ2) higher than that of the thawed late syngamy i.e. 2PN group (11.0%; 123FHs /1121 embryos transferred).

Conclusions: Cryopreserved embryos which had entered syngamy late are as likely to survive thawing, but less likely to resume mitosis and implant post thaw than cryopreserved embryos which had entered syngamy by 23/24 hpi.

CHARACTERIZATION OF E74 LIKE FACTOR 3 IN THE MURINE BLASTOCYST
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The E26 transformation specific (ETS) family of transcription factors is a large family that includes a small number of epithelium specific transcription factors. Elf3 (Ese1) is one of these genes, along with Elf5 (Ese2) and Ese3. Elf3 has been reported in mouse, rat and human organs, including the kidney, small intestine, and uterus and it is thought to have a role in the functional differentiation of these organs. Elf3 mRNA was first detected in the mouse pre-implantation embryo in 2002 and its gene expression appears to increase in the 16 cell embryo and blastocyst compared to previous developmental stages.

Use of reverse transcriptase polymerase chain reaction (RT-PCR) detected two isoforms of Elf3 in the mouse blastocyst. Sequencing showed that these isoforms differ by a 57 base pair insert. This insert is in the Elf3 coding region and translates into an extra 19 amino acids in the pointed domain of the protein. This longer form of the Elf3 mRNA has been previously found in the lung, liver, retina and kidney of the rat. cDNA library analysis detected a similar long isoform of Elf3 in the murine neonate eye. Our work is the first to report the longer Elf3 isoform in the blastocyst.

An in situ hybridisation protocol has been performed with whole blastocysts. Quantitative real time PCR and immunohistochemistry will be used to further investigate Elf3 gene expression and protein location in the mouse blastocyst.
THE INVESTIGATION OF MRNA EXPRESSION OF NUCLEAR IMPORTINS DURING BOVINE PREIMPLANTATION EMBRYOGENESIS.

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At a species-specific point, embryonic genome activation (EGA) occurs; the chromatin-repressed state of the embryo is overcome, and transcription is activated. Embryos produced in vitro are susceptible to developmental arrest at the EGA. Given that functional changes within the nucleus are required for this transition, we hypothesised that production of specific nuclear transport machinery enables the EGA. The importin protein family mediates classical nuclear transport, and this study was designed to assess whether expression of specific importin mRNAs changes at the bovine major EGA (8-16 cell stage).

PCR primers designed from bovine genome sequences were used to amplify partial cDNAs from adult bovine tissue cDNA. PCR product identity was confirmed by DNA sequencing, and these cDNAs were used as positive controls for embryo PCRs. Single embryo amplified cDNA was generated using the SMART™ PCR cDNA Synthesis kit (Clontech) at the following stages: oocyte, 2, 4, 8, and 16-32-cell, morula, day-7 and day-8 blastocysts. Three embryo samples per stage were tested in replicate PCR reactions (n=3). All six importins tested were detected in preimplantation samples but with differing expression patterns. In general, expression appeared more consistent after the EGA than before. Importin β1 appears to belong to the special class of genes whose embryonic transcription begins prior to the major EGA. It was not detected in oocytes and only consistently from the 8-cell stage onwards. Importin 13 and Transportin 2 were detected in some oocytes and 2-cell embryos, and more consistently from the 8-cell stage onwards. Exportin 5, Exportin 1 and Importin β2 were detected in most embryos at all stages. This first description of importin expression during bovine embryogenesis indicates differing requirements for importin family members during preimplantation embryogenesis. Detection of differences in importin expression between embryos at the same stage could provide early indications of differing developmental potential.
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