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PAST ESA OFFICE BEARERS 1958-2007

DATE	PRESIDENT	VICE PRESIDENT	SECRETARY	TREASURER
1958-60	E.Downie		P.Taft	P.Taft
1960-62	C.W.Emmens		K.Harrison	K.Harrison
1962-64	K.Harrison C.W.Emmens (March 63)		I.Thomas	I.Thomas
1964-66	B.Hetzel	V.Trikojus	I.Jarrett	I.Jarrett
1966-68	B.Hudson	V.Trikojus	R.Melick	I.Jarrett
1968-70	P.Taft	R.Cox	R.Melick	I.Jarrett
1970-72	I.Jarrett	K.Ferguson	T.J.Martin	L.Lazarus
1972-74	K.Ferguson	L.Lazarus	R.D.Gordon	L.Lazarus
1974-76	H.G.Burger	J.R.Turtle	S.Posen	C.J.Eastman
1976-78	S.Posen	J.P.Coghlan	P.E.Harding	C.J.Eastman
1978-80	J.P.Coghlan	C.J.Eastman	R.G.Larkins	J.W.Funder
1980-82	C.J.Eastman	J.W.Funder	D.P.Cameron	G.L.Warne
1982-84	J.W.Funder	R.G.Larkins	R.C.Baxter	G.L.Warne
1984-86	R.G.Larkins	D.P.Cameron	R.C.Baxter	D.M.Hurley
1986-88	D.P.Cameron	R.C.Baxter	S.J.Judd	D.M.Hurley
1988-90	R.C.Baxter	S.J.Judd	J.R.Stockigt	D.J.Handelsman
1990-92	J.R.Stockigt	J.A.Eisman	G.W.Tregear	D.J.Handelsman
1992-94	D.J.Handelsman	P.J.Fuller	R.L.Prince	D.J.Topliss
1994-96	P.J.Fuller	R.L.Prince	G.P.Risbridger	D.J.Topliss
1996-98	D.J.Topliss	R.J.Rodgers	G.P.Risbridger	M.S.Lewitt
1998-00	R.J.Rodgers	J.D.Zajac	K.K.Y.Ho B.J.Waddell (May 99)	M.S.Lewitt
2000-02	K.K.Y.Ho	B.J.Waddell	B.Canny	C.Coulter
2002-03	B.Canny	J.D.Zajac	R. Cuneo	C.Coulter
2004-06	J.D. Zajac	L. Bach	M. McLean	V. Clifton
2006-08	L. Bach	M.McLean	V. Clifton	D. Phillips

ESA INTERNATIONAL TRAVEL GRANT

2003	Emma Ball
2004	Gordon Howarth
	Sophie Chan
	Vincenzo Russo
2005	Stuart Ellem
2006	Kevin Pfleger and Erosha Premaratne

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CORPORATE SPONSORS

Lilly Diabetes | Your journey inspires ours.

ESA AWARD SPONSORS

IPSEN Innovation for patient care

SERVIER

NOVARTIS ONCOLOGY

mayne pharma

FUTURE MEETINGS

ESA Seminar
9th -11thMay 2008
Sydney Harbour Marriott Hotel
www.esaseminar.org.au

ESA Clinical Weekend
22nd August – 24th August 2008
Melbourne
www.esaclinicalweekend.org.au

Combined ESA/SRB Annual Scientific Meeting
24th – 27th August 2008
Melbourne Exhibition and Convention Centre
www.esa-srb.org.au

Avandia sustains glycaemic control significantly longer than metformin or sulphonylurea.*1†

ADOPT
A DIABETES OUTCOME PROGRESSION TRIAL

A brighter future^

Avandia®
rosiglitazone maleate

* Glibenclamide (US name: glyburide).

† Primary endpoint: time to monotherapy failure (reconfirmed FPG >10mmol/L). Avandia reduced the risk of monotherapy failure by 32% compared to metformin ($p<0.001$) and 63% compared to sulphonylurea ($p<0.001$). Avandia maintained mean $HbA_{1c} < 7\%$ (secondary endpoint) for 57 months vs 45 months for metformin vs 33 months for glibenclamide. Daily dose titrated to control glycaemia: rosiglitazone (up to 8mg), glibenclamide (up to 15mg) or metformin (up to 2000mg)[1]

^ Sustained reductions in FPG and HbA_{1c} demonstrated in studies of Avandia given once or twice daily as monotherapy or in combination with other antidiabetic agents (sulphonylureas, metformin or insulin)[2]

Please review Product Information before prescribing. **AVANDIA (rosiglitazone maleate). Indications:** Treatment of Type 2 diabetes mellitus. Monotherapy, dual therapy with sulphonylureas, metformin or insulin and in triple therapy with metformin and sulphonylureas, in patients inadequately controlled by diet and exercise. **Contraindications:** Hypersensitivity. **Precautions:** Type 1 diabetes mellitus, premenopausal anovulatory patients with insulin resistance (eg. polycystic ovary syndrome), oedema, New York Heart Association (NYHA) classified heart failure, macular oedema with decreased visual acuity, bone fracture in women*, hepatic dysfunction/impairment, pregnancy (Category B3), lactation, children. **Interactions:** Gemfibrozil, rifampicin. **Adverse Events:** headache, back pain, hyperglycaemia, fatigue, diarrhoea, hypoglycaemia, oedema, anaemia, hypercholesterolaemia, weight gain, hepatic dysfunction, macular oedema, heart failure*, cardiac ischaemia* bone fracture in women* – This is not a full list, for more details refer to full PI. **Dosage:** Initiated at 4mg/day. Can be increased to 8mg/day after 6-8 weeks if greater glycaemic control is required. May be given once or twice daily. Careful dose titration when adding to insulin therapy. **PBS dispensed price:** Avandia 4mg x 28 = $60.25 Avandia 8mg x 28 = $89.92. **References: 1.** Kahn SE et al. N Engl J Med 2006;355:2427-2443 (erratum N Engl J Med 2007;356:1387-8). **2.** Avandia Product Information. Full Disclosure Product Information is available from GlaxoSmithKline Australia Pty Ltd, 1061 Mountain Highway, Boronia, Vic 3155. ABN: 47 100 162 481. Avandia® is a registered trade mark of the GlaxoSmithKline Group of Companies. *Please note change in Product Information. PC0706163 GSKA 6/07

PBS Information: Authority Required. Refer to PBS Schedule for full Authority Required information.

KEITH HARRISON MEMORIAL LECTURES

1964	Kenneth Ferguson
1965	Geoffrey Harris
1973	Albert Renold
1974	Paul Franchimont
1975	William Odell
1976	John Landon
1977	Hugh Niall
1978	Samuel Yen
1979	John Shine
1980	Ronald Swerdloff
1981	Sidney Ingbar
1982	Jens Rehfeld
1983	Philip Lowry
1984	Fernand Labrie
1985	Michael Berridge
1986	Michael Thorner
1987	Lynn Loriaux
1988	Axel Ulrich
1989	Hiroo Imura
1990	Iain McIntyre
1991	Eli Adashi
1992	Jan-Ake Gustafsson
1993	Eberhard Nieschlag
1994	Allen Speigel
1995	Natalie Josso
1996	Gregory Mundy
1997	M.Geoffrey Rosenfeld
1998	Ken Korach
1999	Henry Burger
2000	Pierre Chambon
2001	Jack Martin
2002	George Chrousos
2003	Derek LeRoith
2004	Bruce McEwen
2005	Richard Pestell
2006	William Crowley

NOVARTIS JUNIOR AWARD

The Novartis Junior Award is awarded annually to a member who is a postgraduate student or recent post-doctoral student, for the best original paper at the Annual Scientific Meeting.

1976	Kathryn Rich & Peter Fuller
1977	David Kennaway
1978	David Healy
1979	George Werther
1980	Rebecca Mason
1981	Yvonne Hodgson
1982	David Hurley
1983	Carolyn Scott
1984	David James
1985	Guck Ooi
1986	Marie Ranson
1987	Lora Hutchinson
1988	Vasilious Papadopoulos
1989	David Phillips
1990	Sharon Gargosky
1991	Marie-Christine Keightley & Helen Maclean
1992	Fiona Young
1993	Emma Ball
1994	Vicki Clifton
1995	Michael Downes & Sylvia Lim-Tio
1996	John Walsh
1997	Bu Yeap
1998	Julie Joyner
1999	Renea Jarred & Helena Teede
2000	Jeremy Smith
2001	Stephen Heady
2002	Patrick McManamny
2003	Sophie Chan
2004	Esme Hatchell
2005	Agnes Kovacic & Amy Au
2006	David Macintyre

ESA MAYNE PHARMA BRYAN HUDSON CLINICAL ENDOCRINOLOGY AWARD

The ESA Mayne Pharma Bryan Hudson Clinical Endocrinology Award will recognize the best clinical research presentation at the Annual Scientific Meeting by an active member of the Endocrine Society of Australia early in their career. It will be made on an annual basis.

2004	Sonia Davison
2005	Carolyn Allan
2006	Jui Ho

New dosing option*

Somatuline® autogel® **120mg**
lanreotide

Now with the added convenience of an extended dosing interval

Patients with acromegaly who are well controlled on lanreotide can now be treated with Somatuline Autogel 120 mg every 6–8 weeks[1]

*For patients with acromegaly who are well controlled on lanreotide

References
1. Somatuline Autogel Product Information, 17 August 2006.

PBS Information: Authority required (Section 100). Acromegaly; Symptoms of carcinoid syndrome. Refer to PBS schedule for full information.

Before prescribing please refer to full Product Information, which is available from Ipsen Pty Ltd.
Somatuline® Autogel®: lanreotide as acetate in a pre-filled syringe (60, 90 & 120 mg). **Indications**: the treatment of acromegaly when circulating growth hormone and IGF-1 levels remain abnormal after surgery and/or radiotherapy or in patients who have failed dopamine agonist therapy; the treatment of symptoms of carcinoid syndrome associated with carcinoid tumours. **Contraindications**: lactation; hypersensitivity to lanreotide or related peptides. **Precautions**: may experience hypoglycaemia or hyperglycaemia (monitor blood glucose levels); may reduce gall bladder motility (recommend gall bladder echography); exclude presence of obstructive intestinal tumour; monitor kidney and liver function; may reduce heart rate in patients with an underlying cardiac problem (monitor heart rate). Not recommended for use in children. See full PI for further information. **Adverse Events**: common to very common: fatigue, headache, dizziness, sinus bradycardia, hypoglycaemia or hyperglycaemia, anorexia, diarrhoea, abdominal pain, nausea, vomiting, dyspepsia, flatulence, cholelithiasis, bilirubin increase, injection site reaction. See full PI for further information. **Dose**: Acromegaly: for first time treatment the starting dose is 60 mg every 28 days; for patients previously treated with Somatuline LA every 14, 10 or 7 days, the starting dose is 60 mg, 90 mg or 120 mg respectively every 28 days. Dosage should be adjusted according to GH and/or IGF-1 response. Patients well controlled on lanreotide can be treated with 120 mg every 42–56 days. Carcinoid syndrome: 60 to 120 mg every 28 days, adjusted according to symptomatic relief. **Administration**: deep subcutaneous injection in the superior external quadrant of the buttock. **Storage**: 2°C–8°C. **Date of TGA approval**: 17 August 2006.

For further information about Somatuline Autogel, contact Ipsen Pty Ltd:
T (03) 8544 8100 **F** (03) 9562 5152 **E** info@ipsen.com.au
Suite 6, 40 Montclair Avenue, Glen Waverley, VIC 3150 Australia
All correspondence to: PO Box 820, Glen Waverley, VIC 3150 Australia

IPSEN
Innovation for patient care

Somatuline® autogel®
lanreotide

SERVIER AWARD

The Servier Award is awarded for the best published work in the previous year by a member of the Society within 5 years of award of higher degree.

1991	Sharon Gargosky
1992	Peter Stanton
1993	Janet Martin
1994	Chen Chen
1995	Timothy Crowe
1996	Jun-Ping Lui
1997	Liza O'Donnell
1998	Stephen Twigg
1999	Dan Lee
2000	Fraser Rogerson
2001	Karen Kroeger
2002	Susan Fanayan
2003	Jenny Gunton
2004	Peter Liu
2005	Simon Chu
2006	Renea Taylor

HONORARY LIFE MEMBERS

Prof Robert Baxter
Dr A.W. Blackshaw
Dr H.D. Breidahl
Prof James B. Brown
Prof Henry G. Burger
Dr R.A. Burston
Prof Donald P. Cameron
Prof John P. Coghlan
Prof Alex Cohen
Dr Ron I. Cox
Prof David De Krester
Prof C.J. Eastman AM
Dr K.A. Ferguson
Prof John W. Funder
Prof R.D. Gordon
Dr Ian B. Hales
Dr Philip Harding
Prof Basil Hetzel
Dr Brian Hirschfeld
Dr Ivan G. Jarrett
A/Prof Stephen Judd
Prof Richard G. Larkins
Prof Leslie Lazarus
Dr T.B. Lynch
Prof T. John Martin
Dr Len Martin
Dr F.I.R Martin
Dr Ian C.A. Martin
Prof Solomon Posen
Prof Marilyn Renfree
Prof T.J. Robinson
Prof Alfred W. Steinbeck
Prof Jim Stockigt
Dr Ian D. Thomas
Emeritus Prof John R. Turtle
Dr A.L. Wallace
Prof Marelyn Wintour-Coghlan
Dr K.N. Wynne

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Christchurch Executive Council (2007 - Present)
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Graham Barrell (Deputy President)
Margaret Evans (Secretary)
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Allan Pearson (Communicating member, Hamilton)
John Delahunt (Communicating member, Wellington)
Greg Anderson (Communicating member, Dunedin)
John Cockrem (Communicating member, Palmerston North)

2007 NZSE Nancy Sirett Lecturer

Jeff Keelan "Placental mediators of pregnancy and parturition"

CONFERENCE ORGANISING COMMITTEES

The Local Organising Committee
SRB: Peter Hurst, Jean Fleming, Helen Nicholson, Darryl Russell
ESA: Warrick Inder, Helen MacLean
NZSE: Dave Gratten, Greg Anderson, Penny Hunt

ESA Program Organising Committee
Helen MacLean (chair), Warrick Inder, Mathis Grossmann, Carolyn Allan, Vince Russo, Brian Oldfield, Greg Anderson

Conference 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

Society Secretariat - Endocrine Society of Australia
Ivone Johnson
145 Macquarie Street
SYDNEY NSW 2000
Ph: 02 9256 5405 Fax: 02 9251 8174
Email: esa@racp.edu.au
Website: www.endocrinesociety.org.au

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Diagnostic Systems Laboratories

AWARDS

Novartis Oncology

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Serono

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Servier

Mayne Pharma

IPSEN

INVITED SPEAKER PROFILES 2007

Iain J Clarke (SRB MLA Plenary Lecturer)
Prof Clarke did his agricultural degrees at Massey University, Palmerston North, New Zealand and his PhD at Edinburgh University. His PhD work set the scene for prenatal androgenisation as a model of polycystic ovarian disease. He moved to Australia in 1977 to work on Clover Disease in sheep, investigating effects of the now named 'endocrine disruptors'. He took a position at Prince Henry's Institute in 1999 and remained there until 2005, experiencing the rigor of the NHMRC Fellowship Scheme for 2 decades. He moved to Monash University in 2005 and was took up the post of Head of Dept Physiology in 2007. Professor Clarke's work is characterised by focus on whole animal models of reproduction, growth, lactation, stress and metabolic function. He has published almost 400 research papers in these areas and has received a Senior Fulbright Award, The Woodward Prize for Neuroscience (Australia) and the British Endocrine Society Asia and Oceania Medal. His current research is focused on the areas of reproduction, stress, appetite and energy balance. Notable scientific landmarks of his career have been the establishment of the hypophysial portal access model and the hypothalamo-pituitary disconnection, in collaboration with his close and enduring friend, Mr James Cummins (neurosurgeon). The former provided the first insight into real-time *in vivo* secretions of the hypothalamus and the latter has endured as an *in vivo* isolated pituitary model. He has enjoyed a marriage of 30 years and has two daughters. His only unfulfilled objective is that he never made it back to his homeland!

Takashi Kadowaki (ESA Japan-Australia Lecturer)
Prof Takashi Kadowaki, MD, PhD, is Professor in the Department of Metabolic Diseases, Graduate School of Medicine, The University of Tokyo, and Vice-Director of Tokyo University Hospital. He trained in medicine at the Tokyo University Medical School, and after his clinical Fellowship in Diabetes, was a Visiting Fellow in the Biochemistry and Molecular Pathophysiology Section, Diabetes Branch, NIDDK at NIH. His research interests focus on understanding the molecular mechanisms of type 2 diabetes, insulin resistance and obesity. Prof Kadowaki's research encompass seminal studies including the identification of adiponectin and its receptors, and has been published in Nature, Science, Nature Genetics, Molecular Cell and the New England Journal of Medicine. Prof Kaodwaki is the recipient of the Sankyo Takamine Memorial Award, Hagedorn Award of the Japan Diabetes Society and the Erwin von Baelz prize. He is on the editorial boards of journals including Diabetes Care, Endocrinology, Current Diabetes Reviews, the Journal of Endocrine Genetics and the Journal of Clinical Investigation.

Gerard Karsenty (ESA Harrison Memorial Lecturer)
Prof Gerard Karsenty is the Paul A Marks Professor and Chair of the Department of Genetics and Development, Columbia University, NY. He completed his MD and PhD at the Medical School Paris, before moving to the US in 1985, where he worked at the University of Texas and Baylor College of Medicine prior to his move to New York in 2006. His research focuses on elucidation of the mechanisms accounting for the differentiation and the function of the osteoblast, linking the genes and pathways studied to human disease and their treatment. Prof Karsenty's research group has made a number of seminal discoveries in the field of bone and metabolism, including identifying Runx2 as the master gene of osteoblast differentiation and showing that haploinsufficiency at the *Runx2* locus causes cleidocranial dysplasia, identifying ATF4 acting as a differentiation factor and showing that loss of ATF4 activity causes Coffin-Lowry Syndrome, discovering two of only three known osteoblast-specific transcription factors, and postulating based on clinical observations that bone mass, body weight and reproduction should be controlled by the same hormones, leading to the demonstration that leptin regulates bone mass. Prof Karsenty is editor of a number of journals including Journal of Cell Biology, Developmental Cell, Molecular and Cellular Biology and Cell Metabolism. His research has been recognised by numerous awards, including the Drieu-Cholet Award from The National Academy of Medicine of France, the Louis Avioli Founders Award from ASBMR and the D Harold Copp Award from IBMS.

Jeff Keelan (ESA/NZSE Nancy Sirett Lecturer)
Jeff Keelan gained his PhD from the Department of Obstetrics & Gynaecology, University of Auckland in 1994 after studying the production and regulation of inhibins and activins in the human placenta. He subsequently joined the department of Pharmacology and Clinical Pharmacology to undertake postdoctoral studies with Professor Murray Mitchell investigating inflammatory mediators of parturition in the context of preterm labour and delivery. He was appointed Senior Lecturer in the same department in 2001, where he expanded his interests to include placental pharmacology and drug transport, and was a founding member of the Liggins Institute when it was formed in 2001. In 2006 he was made leader of the Institute's Pregnancy and Parturition group and was the recipient of the first March of Dimes grant to be funded in New Zealand. He is has recently taken up an Associate Professorship at the University of Western Australia, funded by the Women and Infants Research Foundation, and is continuing his studies into inflammation in pregnancy, placental drug transfer, placental apoptosis and mediators of term and preterm labour. He is the author of over 80 research papers, reviews and book chapters, has supervised 18 postgraduate students, and is a reviewer for over 20 journals and 14 funding bodies. He is President of the Australian and New Zealand Placental Research Association (ANZPRA) and a member of the SRB, NZSE, SGI and RSNZ.

Martin Matzuk (SRB Founders Lecturer)
Martin's research interests focus on the molecular genetics and cell biology of germ cell differentiation and maturation. His group has been at the forefront of a revolution in thought on the endowment of maternal effect genes that control embryogenesis. His group have developed genetic models to decipher the crosstalk of TGF superfamily (e.g., activin, inhibin, BMP, and GDF) signalling pathways regulating folliculogenesis and testis development and leading to mature germ cell production. In addition to identifying the molecular processes that direct maturation of sperm as well as maturation and ovulation of the oocyte they discovered factors that control embryo development following fertilization and preceding zygote genome activation. Martin is the *Stuart A. Wallace* Chair of the Department of Pathology, Baylor College of Medicine and Professor in the Departments of Pathology, Molecular & Cellular Biology, and Molecular & Human Genetics at Baylor College of Medicine, Houston Texas, USA.

Robert J Smith (ESA Pincus Taft Lecturer)
Dr. Robert Smith completed undergraduate studies in biochemistry and then obtained his M.D. degree from Harvard Medical School. He is certified in Internal Medicine and in Endocrinology, Diabetes, and Metabolism. After many years on the faculty of Harvard Medical School, he moved to Brown University in 2000 as Director of Endocrinology and founding Director of the Hallett Center for Diabetes and Endocrinology. His honors have included an American Medical Association Goldberger Fellowship Research Award, the Harvard Division of Medical Ethics Responsible Conduct of Research Award, and appointment to the Howard Hughes Medical Institute. In addition to his administrative and clinical responsibilities, Dr. Smith directs a basic and clinical research program, and has published extensively on the role of altered hormone signaling in diabetes mellitus, genetic and acquired growth disorders, and catabolic states associated with severe illness. His work has a strong focus on the molecular mechanisms of cellular signaling by insulin and insulin-like growth factors and the changes in these signaling pathways that lead to human disease. As a component of his current studies, he is investigating novel proteins involved in both diabetes and neurodegenerative diseases.

PROTOS®

strontium ranelate

PBS LISTED APRIL 1[1]*

FIRST OF A NEW CLASS[1,2]

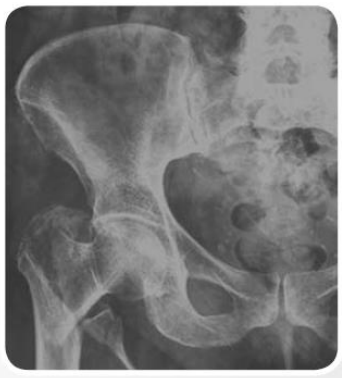
FOR POSTMENOPAUSAL OSTEOPOROSIS*

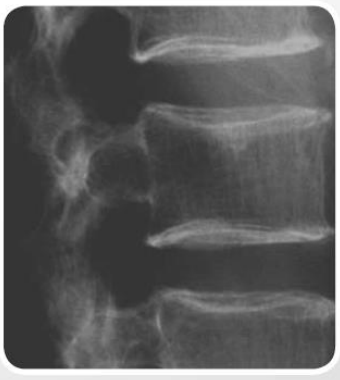
Dual Acting Bone Agent[1,2,3]

*Increases bone formation **AND** decreases bone resorption*[2,3]

*Protects against vertebral **AND** non-vertebral fractures*[3,4]

*Protects patients with **AND** without previous fracture*[*3,4,5]





PROTOS® Abridged Product Information. Before prescribing, please refer to Approved Product Information. Indications: treatment of postmenopausal osteoporosis to reduce the risk of fracture. **Contraindications:** known hypersensitivity to strontium ranelate or to any of the excipients. Severe renal impairment. **Precautions:** use with caution in patients at increased risk of VTE, including patients with a past history of VTE. PROTOS contains aspartame, a source of phenylalanine, which may be harmful for people with phenylketonuria. Category B3. **Discontinue if serious allergic reaction**.** Do not use in pregnancy. **Interactions:** should preferably be taken ≥ 2 hours after: - food, milk, milk products, or medicines containing calcium, which may reduce bioavailability. - tetracycline, as may reduce tertracycline absorption. Concomitant bisphosphonate treatment is not recommended. No known interaction with oral vitamin D. **Adverse Reactions:** nausea, diarrhoea, headache, dermatitis, eczema, loose stools. Less common reactions: consult Approved Product Information. **Dosage and Administration:** one 2g sachet once daily by mouth preferably at bedtime or ≥ 2 hours after food. No dosage adjustment required in the very elderly, mild to moderate renal impairment, or hepatic impairment. **Presentation:** PROTOS 2g sachets contain 2g strontium ranelate as a yellow powder. Boxes contain 7 or 28 sachets. **Date of Preparation:** 21 June 2005. PBS Dispensed Price – **May 2007**: $52.29, 1 + 5 repeats. Full Approved PI is available on request from Servier Laboratories (Australia) Pty. Ltd. 8 Cato Street Hawthorn, VIC 3122. Customer Service (Toll Free) 1800 33 1675.

1. MIMS issue no.2 2007 2. Australian Approved Product Information 2005. 3. Meunier PJ et al. *N Engl J Med 2004*; 350:459-68. 4. Reginster J.Y et al. *J of Clin Endoc Metab 2005*; 90(5):2816-2822. 5. Roux C et al. *JBMR 2006*; 21(4) 536-542.

* PBS listed for secondary prevention of fracture **** Please note changes in Product Information**

6/07 TAC3163

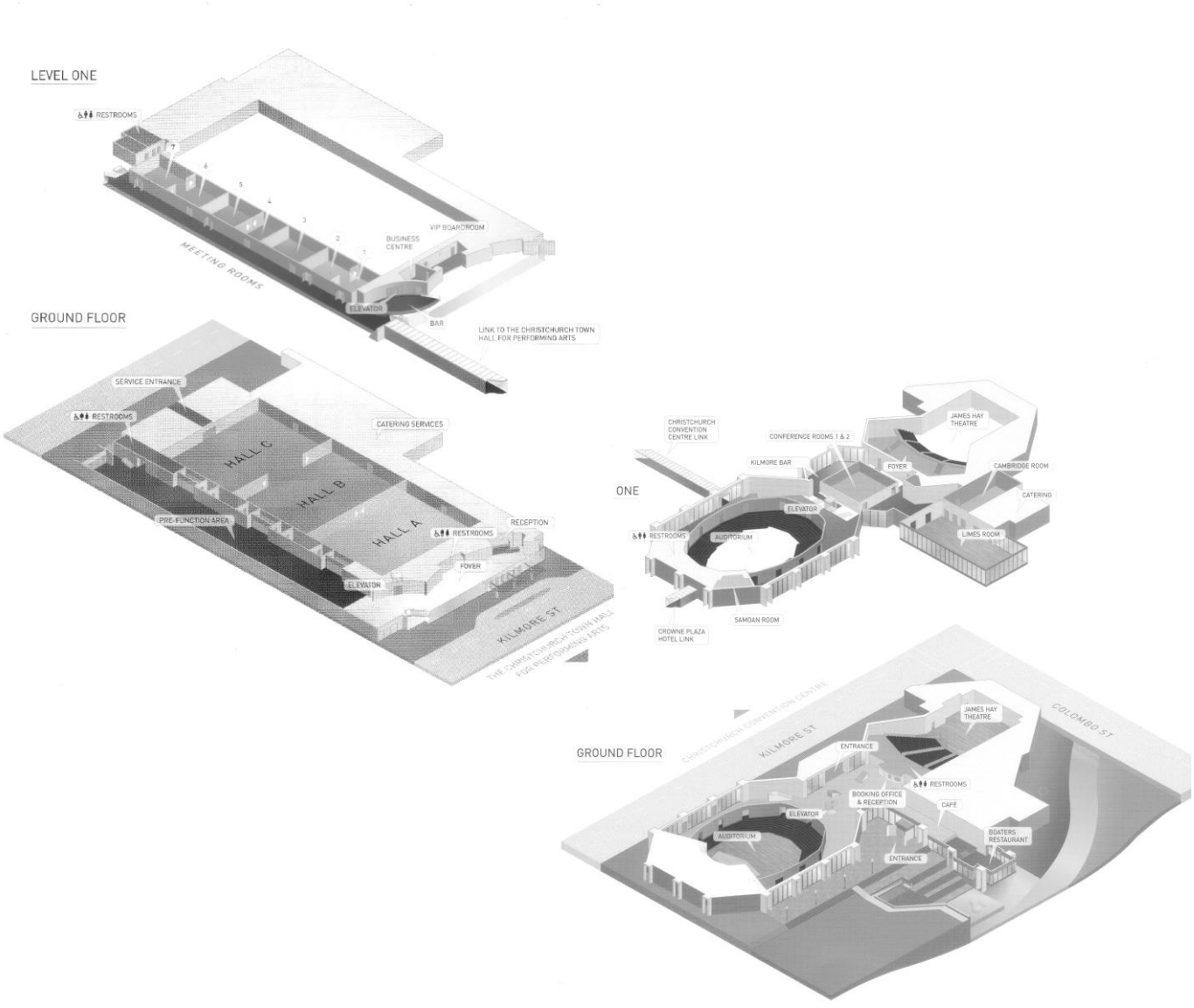
PBS Information: Authority required (STREAMLINED). Refer to PBS Schedule for full authority information.

INFORMATION FOR DELEGATES & PRESENTERS

Venue Location
Christchurch Convention Centre
95 Kilmore Street
Christchurch, New Zealand 8141

The Christchurch Convention Centre is New Zealand's largest purpose-built convention venue. Situated in the heart of Christchurch's CBD, this iconic Canterbury landmark is surrounded by deluxe accommodation and is only 15 minutes from Christchurch International Airport. Its unique and contemporary architecture boasts a 10-metre high glass façade and is connected by air bridge to the Christchurch Town Hall for Performing Arts and one of the city's leading hotels.

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 the first floor entrance of the Christchurch 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.

REGISTRATION DESK PHONE NUMBER 0(+64) 3 363 3370

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.

Poster Viewing
Delegates with posters can find the correct position for their poster by finding the appropriate abstract number on the display panels in Convention Centre's upstairs foyer (adjacent to the exhibition area). The program provides your abstract number which is how you find you placement position. Posters can remain on display all of Monday and Tuesday and must be removed after the day's sessions on Tuesday. During formal poster discussions, the presenters should be present at their poster to answer questions and meet colleagues with similar research interests.

Social Functions

- The **Welcome Function** is in the Christchurch 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 Annies Wine Bar. Delegates 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 Limes Room, Town Hall. Pre-dinner drinks will be served from 7:00pm for a 7:30pm start. Dress is neat casual. This is a ticketed function and they must be purchased in advance.

The Trade Passbook Competition - Amongst delegate's registration papers is a "Trade Pass Book" entry form. The form has spaces for the stamp or signature of each of the trade exhibitors. Once you have collected 15 stamps or signatures, place your completed form in the entry box at the registration desk by the end of afternoon tea on the Tuesday. The prize for the first completed entry form drawn from the box is a NZ wine pack donated by ASN Events. *Trade representatives are not eligible to enter the competition.

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

Smoking - is not permitted in the venue.

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

Message Board - will be available at the registration desk.

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.

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Sunday, 2 September 2007

SRB Workshop - Scientific Journal Publishing

2:00 PM - 3:30 PM — James Hay

Sharon Mortimer

Scientific Journal Publishing *abs#001*

Afternoon Tea

3:30 PM - 4:00 PM — James Hay

SRB Symposium - Gamete Development and Maturation

4:00 PM - 5:30 PM — James Hay

4:00pm **Kate Loveland**
Out of control: The transforming growth factor-β superfamily in early spermatogenesis and its potential relevance to testicular dysgenesis *abs#002*

4:30pm **Grant Montgomery**
Genetic variants in GDF9 and BMP15 in mothers of dizygotic twins *abs#003*

5:00pm **Robert Gilchrist**
Oocyte-secreted factor regulation of follicle and oocyte maturation. *abs#004*

SRB-RCRH Excellence in Reproductive Biology Research Award Lecture

5:30 PM - 6:00 PM — James Hay

Chair: Michael Holland

Eileen McLaughlin

Under attack: germ cell defences against environmental toxins? *abs#005*

ESA / SRB / NZSE Welcome Function

6:00 PM - 7:30 PM — Limes Room

Sponsored by

ESA / SRB Women in Endocrinology Function

7:00 PM - 8:00 PM — Conference 2

Sponsored by

Monday, 3 September 2007

ESA Taft Plenary Lecture

8:30 AM - 9:30 AM — James Hay

Chair: Mark McLean

Robert J Smith

A Molecular Journey from Insulin and IGFs to Neurodegenerative Disease *abs#006*

SRB Orals 1 Ovarian folliculogenesis

8:30 AM - 9:45 AM — Limes Room

Chairs: Jock Findlay and Kylie Dunning

8:30am **Peter Smith**
Developmental programming: effects of prenatal testosterone excess on follicular recruitment and depletion *abs#007*

8:40am **Helen Irving-Rodgers**
Developmental competence of oocytes relates to ovarian follicular basal lamina phenotype *abs#008*

8:50am **Carolina Vinoles**
The static and immediate effects of nutrition act in synergy to increase follicle development in Merino ewes *abs#009*

9:00am **Lisa Haydon**
Expression of Connexins 37, 40, and 43 in the ovaries of the brushtail possum, sheep, rat, and rabbit *abs#011*

9:10am **Rebecca Craythorn**
Abnormalities in the female reproductive tract of follistatin null mice expressing a human follistatin-315 transgene *abs#012*

SRB Orals 2 - Growth factors and signalling

8:30 AM - 9:45 AM — Cambridge

Chair: Laura Parry

8:30am **Julia Young**
In silico transcriptional profiling of differentially expressed genes in response to bone morphogenetic proteins *abs#013*

8:40am **Eva Dimitriadis**
Interleukin 11 and leukemia inhibitory factor regulate the adhesion of human enometrial epithelial cells *abs#014*

8:50am **Shirley Martin**
Creating a chimeric TGFβ family receptor to study receptor function *abs#015*

9:00am **Maree Bilandzic**
Loss of TGF-beta sensitivity linked to low betaglycan expression in granulosa cell tumour lines. *abs#016*

9:10am **Amanda Sferruzzi-Perri**
Insulin-like growth factor-II promotes placental functional development and fetal growth via the type 2 IGF receptor *abs#017*

9:20am **Claire Roberts**
Complex interactions of IGF-II, uPA uPA receptor and plasminogen with oxygen in trophoblast invasion *abs#018*

ESA Servier Award

9:30 AM - 9:45 AM — James Hay

Chairs: David Phillips and Chen Chen

Morning Tea

9:45 AM - 10:15 AM — Exhibition Hall

ESA Novartis Junior Investigator Award Finalists

10:15 AM - 12:00 PM James Hay

Chairs: Mark McLean and Jeremy Smith

10:15am **Sean (Seung-Kwon) Yang**
Involvement of TTX-resistant Na^+ currents and protein kinase C on primary cultured somatotropes from GFP-GH transgenic mice in the effect of GHRH *abs#019*

10:30am **Michael Pearen**
Beta-adrenergic regulation of norphan nuclear receptor signalling: insights into the control of metabolism *abs#020*

10:45am **Niroshani Pathirage**
Molecular Basis of aromatase over-expression in Ovarian Granulosa Cell Tumours *abs#021*

11:00am **Marianne Elston**
Wnt pathway inhibitors are strongly down-regulated in pituitary tumours *abs#022*

11:15am **Wee-Ching Kong**
MicroRNAs are differentially expressed between mid and late gestation in the mouse placenta. *abs#023*

11:30am **Jyothsna Rao**
Adiponectin acutely increases intracellular calcium and insulin secretion in Min6 cells *abs#024*

NZSE Student Award Finalists

10:15 AM - 12:00 PM Conference 1

Chairs: John Evans, Graham Barrell

10:15am **Charisma Dhaliwal**
Diet-Induced Obesity and Prenatal Undernutrition Lead to Central Leptin Resistance by Different Mechanisms *abs#025*

10:30am **Emma Kay**
A comparison of mouse and human MRAPs: accessory proteins for the human melanocortin receptor 2 in HEK293 cells *abs#026*

10:45am **Lachlan Pearson**
Regulation of endothelin-1 in human endothelial cells by sex steroids and angiotensin II. *abs#027*

11:00am **Hana-Lee Relf**
Does RFRP-3 suppress pulsatile LH secretion in the rat? *abs#028*

SRB Orals 3 - Pregnancy and fetal development

10:15 AM - 12:00 PM Limes Room

Chairs: Jeff Kellan and Guiying Nie

10:15am **Karensa Menzies**
Role for folate to increase milk protein production *abs#029*

10:25am **Ellen Menkhorst**
The relationship between plasma progesterone, conceptus development and gestation length in the Stripe-faced Dunnart, *Sminthopsis macroura*. *abs#030*

10:35am **Adam Morrissey**
Milk yield in ewes during mid- to late lactation is not affected by pregnancy *abs#031*

10:45am **Alison Care**
A Novel Mouse Model for Endometrial Macrophage Evaluation in Early Pregnancy *abs#032*

10:55am **Peter Mark**
Placental P-glycoprotein (*Abcb1*) expression is reduced by glucocorticoids during late gestation in the rat *abs#033*

11:05am **David Sharkey**
Transforming growth factor beta is a major cervical signaling factor in human seminal plasma *abs#034*

11:15am **Neil Gude**
The angiogenesis inhibitor calreticulin is increased in maternal blood with pregnancy and pre-eclampsia *abs#035*

11:25am **Hitomi Nakamura**
Macrophage Regulation of Embryo Adhesion Molecule Expression in Human Endometrial Epithelial

Cells *abs#036*

11:35am **Santwona Bhattu**
Implantation rates in mouse following treatment with Lipiodol *abs#037*

11:45am **Lenka Vodstrcil**
The decrease in myometrial relaxin receptor (Lgr7) expression at the end of gestation in the rat is driven by the fetal-placental unit and not maternal progesterone *abs#038*

SRB Orals 4 - Comparitive Reproductive Biology

10:15 AM - 12:00 PM Cambridge
Chairs: Geoff Shaw & Sue Jones

10:15am **Brandon Menzies**
Age and sex specific regulation of the growth axis during development in the tammar wallaby: A model to study mammalian fetal growth and development *abs#039*

10:25am **Andrew Pask**
Marsupial WT1 has a Novel Isoform and is Expressed in both Somatic and Germ Cells in the Developing Ovary and Testis *abs#040*

10:35am **Janet Crawford**
Does prolactin play a role in reproduction in the brushtail possum? *abs#041*

10:45am **Alison Cree**
Maternal influence on sex in a live-bearing gekkotan lizard from southern New Zealand *abs#042*

10:55am **John Clulow**
Amphibian Genome Cryobanking – Success in Sperm Cryopreservation but the Block to Embryo Cryopreservation Remains. *abs#043*

11:05am **Natasha Czarny**
Preservation of spermatozoa from dasyurid marsupials *abs#044*

11:15am **Annelie Moberg**
Cloning and Functional Analysis of Proacrosin from a Marsupial, the Tammar Wallaby (Macropus eugenii) *abs#045*

11:25am **Jinwei Chung**
Patterning factors in the developing gonad of the marsupial *abs#046*

11:35am **Jane Fenelon**
Expression of EGF and EGF-R in the endometrium during entry into and reactivation from embryonic diapause in the tammar wallaby, *Macropus eugenii.* *abs#047*

11:45am **Natalie Calatayud**
The effects of oestrogen on sexual differentiation in a marsupial, Macropus eugenii *abs#048*

SRB Founders Lecture

12:00 PM - 1:00 PM James Hay

Martin Matzuk
Insights into Reproductive Biology *abs#049*

ESA Monday Poster Session

12:00 PM - 1:00 PM Upstairs Foyer
See listing at end of program
Odd numbered abstracts to be attended
Topics: Female Reproduction and Pregnancy; Male Reproduction; Metabolism and Obesity; Clinical I

ESA Clinical - Meet the Expert 1

12:30 PM - 1:30 PM Conference 2
Chair: Carolyn Allan

Robert McLachlan
Androgen Deficiency *abs#050*

Lunch

1:00 PM - 2:00 PM — Exhibition Hall

Sponsored by

ANZPRA AGM (during lunch)

1:15 PM - 2:00 PM — Conference 1

SRB / ANZPRA Symposium - Non-Genetic Maternal and Paternal Influences on Fetal Outcomes

2:00 PM – 4:00 PM — Conference 1

2:00pm **Larry Chamley**
Maternal responses to deported trophoblasts *abs#051*

2:30pm **Melinda Jasper**
Male seminal fluid regulation of female tract receptivity for embryo implantation *abs#052*

3:00pm **Valerie Grant**
Is there a maternal influence on sex allocation in mammals? *abs#053*

3:30pm **Emma Whitelaw**
Epigenetic modifiers show paternal effects in the mouse *abs#054*

ESA / SRB Joint Orals - Male Reproduction

2:00 PM – 4:00 PM — Cambridge

Chairs: Charles Allan and Kate Loveland

2 :00pm **Kyriakos Pratis**
Identifying novel contraceptive targets: transcriptional profiling of specific stages of rat spermatogenesis during hormone suppression *abs#055*

2:15pm **Wendy Ingman**
An acute macrophage depletion model reveals a role for macrophages in regulation of testicular steroidogenesis in vivo *abs#056*

2:30pm **Prue Cowin**
Transient *in utero* exposure to the endocrine disruptor Vinclozolin induces inflammation and atrophy in the post- but not pre-pubertal prostate *abs#057*

2:45pm **Gabrielle Wilson**
Genetic and functional analysis of PACRG, a novel protein associated with human male infertility *abs#058*

3:00pm **Mark Hedger**
Identifying a role for inflammatory intermediates in germ cell-Sertoli cell communication *abs#059*

3:15pm **Ulla Simanainen**
Male mice with androgen receptor disruption targeting sex accessory organs are subfertile *abs#060*

3:30pm **Mark McCabe**
Effect of gonadotrophin suppression on testicular tight junctions *abs#061*

3:45pm **Duangporn Jamsai**
The role of gametogenetin (Ggn) in spermatogenesis and pre-implantation-embryo development *abs#062*

ESA Clinical - Case Reports

2:00 PM - 4:00 PM — Conference 2

Chair: Mathis Grossman, Ann McCormack

2:00pm **Paul Lee**
Successful treatment of inappropriate hyporeninaemic hypoaldosteronism in cerebral salt wasting secondary to bilateral subdural haematomas with fludrocortisone *abs#063*

2:15pm **Elif Ekinci**
Carbimazole-induced Agranulocytosis *abs#064*

2:30pm **Paul Myhill**
Granulomatous disease as a cause of severe hypercalcaemia. *abs#065*

2:45pm **Bidhu Mohapatra**
Primary hyperparathyroidism can be difficult to diagnose - two unusual cases *abs#066*

3:00pm **Florence Law**
Bilateral adrenal infarction due to heparin-induced-thrombocytopaenia-thrombosis-syndrome (HITTS) *abs#067*

3:15pm **Phillip Wong**
A Case of Regional Osteoporosis *abs#068*

3:30pm **Sarina Lim**
Aseptic meningitis associated with lymphocytic hypophysitis and thyroiditis *abs#069*

3:45pm **Kathryn Hackman**
An Incidental Finding - Phaeochromocytoma in a Patient with Von Recklinghausen's Disease (Neurofibromatosis Type 1) *abs#070*

ESA Orals – Nuclear Hormone Receptor Action

2:00 PM - 4:00 PM James Hay

Chairs: Timothy Cole and Christine Clarke

2:00pm **Wayne Tilley**
Control of androgen receptor signaling in prostate cancer by the cochaperone small glutamine-rich tetratricopeptide repeat containing protein alpha *abs#071*

2:15pm **Christine Clarke**
Rapid movement of PR into subnuclear foci depends on association of PR with chromatin *abs#072*

2:30pm **Ashwini Chand**
Identification of novel pharmacological antagonists for LRH-1 *abs#073*

2:45pm **Kesha Rana**
Skeletal muscle as a target for regulation of fat mass and metabolism in male androgen receptor knockout mice *abs#074*

3:00pm **Carolyn Mitchell**
Glucocorticoid receptorα, pCREB-1 and CBP binding on the prostaglandin endoperoxide H synthase (PGHS-2) promoter of term amnion *in vivo*. *abs#075*

3:15pm **George Muscat**
RORalpha, an orphan NR modulator of metabolism in the liver and peripheral metabolic tissues. *abs#076*

3:30pm **Helen MacLean**
Characterisation of myoblast- and myofibre-specific androgen receptor knockout mice to identify mechanisms of anabolic actions of androgens in skeletal muscle *abs#077*

3:45pm **Timothy Cole**
Disrupted glucocorticoid and cAMP signaling via CREB cause distinct but overlapping phenotypes in the developing mammalian lung *abs#078*

ESA Orals - HPA

2:00 PM - 4:00 PM Limes Room

Chairs: Warrick Inder and Jane Ellis

2:00pm **John Wentworth**
Prospective evaluation of a protocol for reduced glucocorticoid replacement in transsphenoidal pituitary adenomectomy for non-Cushing's tumours: prophylactic glucocorticoid replacement is unnecessary in low-risk cases. *abs#079*

2:15pm **Lucia Gagliardi**
Vasopressin-sensitive ACTH-Independent Macronodular Adrenal Hyperplasia (AIMAH): a rare cause of Cushing's syndrome. Clinical and genetic studies of a large South Australian kindred. *abs#080*

2:30pm **Drusilla Mason**
Effect of corticotropin-releasing hormone (CRH) and cortisol on desensitization of the adrenocorticotropin (ACTH) response to arginine vasopressin (AVP) in ovine anterior pituitary cells *abs#081*

2:45pm **Christina Jang**
Skeletal Muscle 11β Hydroxysteroid Dehydrogenase type 1 is upregulated following Elective Abdominal Surgery *abs#082*

3:00pm **Paul Lee**
Factors determining inadequate hypoglycaemia during insulin tolerance testing after pituitary surgery *abs#083*

3:15pm **Yao Wang**
Inhibin A blocks growth/differentiation factor (GDF)-9 action in adrenocortical cancer (AC) cells *abs#084*

3:30pm **Chris Charles**
Arterio-Venous Trans-Organ Sampling in Normal and Heart Failure Sheep: Determining the Source of Circulating Hormones. *abs#085*

3:45pm **Belinda Henry**
Cortisol effects on food intake, leptin and adiposity are dependent on season in the ewe *abs#086*

Afternoon Tea - ESA
4:00 PM - 4:15 PM Exhibition Hall

SRB Orals 5 - Endocrine regulation of reproductive function
4:15 PM - 6:15 PM Conference 1
Chair: Chris Scott

4:15pm **Gerard Karsenty**
Reciprocal Regulation of Bone Metabolisms *abs#087*

4:25pm **Christopher Scott**
The effect of testosterone & season on preproorexin mrna expression in the hypothalamus of the ram *abs#088*

4:35pm **Penny Back**
Effect of concentrate feeding on insulin response postpartum in grazing dairy cows *abs#089*

4:45pm **Jean Fleming**
Effects of high dietary phytoestrogen intake on ovarian inclusion cyst formation in incessantly ovulated CD-1 mice *abs#090*

4:55m **Bree Pierce**
Stress-like levels of cortisol do not affect the onset or duration of oestrus in ewes *abs#091*

5:05pm **Seng Liew**
Effects of letrozole on the phenotype of adult AROM+ female mice *abs#092*

5:15pm **Penny Hawken**
Is neurogenesis involved in the endocrine response of ewes to rams? *abs#093*

5:25pm **Traute Flatscher-Bader**
Regional expression of kisspeptin, oestrogen receptor alpha and GnRH mRNA within the hypothalamus of the cow *abs#094*

5:35pm **Daniel Inglis**
Development of an improved E-screen *abs#095*

5:45pm **Shalini Panwar**
In vivo effects of immunization against leptin on ovarian function in pre-pubertal female mice *abs#096*

SRB/ANZPRA Orals 6 - Implantation, placental development and function
4:15 PM - 6:15 PM Conference 2
Chair: Larry Chamley and Amanda Sferruzi-Perri

4:15pm **Qi Chen**
The role of IL-6 in causes spreading of endothelial cell dysfunction preeclampsia. *abs#097*

4:25pm **Padma Murthi**
Homeobox gene *HEX* expression is increased in human idiopathic fetal growth restriction. *abs#098*

4:35pm **Rosemary Keogh**
Synthesis of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) by extravillous trophoblast is regulated by p38 mitogen-activated protein kinase and Janus kinase 1 *abs#099*

4:45pm **Kathryn Askelund**
The effect of oxygen and tumor necrosis factor on the number of trophoblasts shed from the human placenta *in vitro* *abs#100*

4:55pm **Denise Furness**
Folate nutrigenomics and maternal DNA damage in uteroplacental insufficiency *abs#101*

5:05pm **Devaki de Silva**
The effects of NF-kB inhibitors on pro-inflammatory cytokine gene expression and apoptosis in human choriodecidual cells *abs#102*

5:15pm **Guiying Nie**
Placental production and secretion profiles of the newly identified serine protease HtrA3 throughout human pregnancy and association with placental insufficiency *abs#103*

5:25pm **Lloyd White**
A comparison of gene expressions between first trimester, term and preeclamptic human placentae *abs#104*

5:35pm **Kirsten McTavish**
Rising FSH in ageing transgenic FSH mice accelerates female reproductive failure: oocyte, embryo and/or uterus? *abs#105*

5:45pm **Kirsty Pringle**
Effect of IGF-II, uPA and Plasminogen in Combination on Blastocyst Development *abs#106*

ESA Basic Symposium - Growth Hormone, Insulin and IGF Signalling

4:15 PM - 6:15 PM James Hay

Chairs: George Werther and Rob Baxter **Sponsored by** Avandamet® rosiglitazone maleate/metformin HCl see boxed warning

4:15pm **Michael Waters**
Insights into growth hormone action from mouse models *abs#107*

4:45pm **Robert Smith**
IGF Signaling through an IGF Receptor-Bound Transcription Factor *abs#108*

5:15pm **Peter Lobie**
Oncogenic Potential Of Autocrine Human Growth Hormone *abs#109*

5:45pm **Janet Martin**
Cell signalling by IGFBP-3: How do you do what you do to me? *abs#110*

ESA Basic Symposium - Neurohypophyseal Hormones, Physiology & Behaviour

4:15 PM - 6:15 PM Limes Room

Chair: Greg Anderson, John Evans **Sponsored by** NOVARTIS ONCOLOGY

4:15pm **Colin Brown**
Cutting out the middle-man - autocrine inhibition of vasopressin secretion by central neuropeptide release *abs#111*

4:45pm **C Carter**
Molecules and Monogamy: What's Love Got To Do With It? *abs#112*

5:15pm **Michael McKinley**
Keeping a cool head: CNS integration of hormonal and behavioural osmoregulatory and thermoregulatory mechanisms. *abs#113*

5:45pm **Rick Jackson**
The Neurophysiology of Love *abs#114*

ESA Clinical / Basic Symposium - Stem Cells and Therapeutic Cloning

4:15 PM - 6:15 PM Cambridge

Chair: Peter Leedman **Sponsored by** SERVIER

4:15pm **Megan Munsie**
Overview of Therapeutic Cloning and Stem Cell Therapy – The Science Behind the Headlines *abs#115*

4:45pm **Bernard Tuch**
Use of Human Embryonic Stem Cells to Develop Novel Therapies for Type 1 Diabetes *abs#116*

5:15pm **Chris O'Neill**
Some speed bumps on the road to cell therapies *abs#117*

5:45pm **Rodney Rietze**
Growth hormone receptor signalling regulates the prevalence of adult neural stem cells *abs#118*

SRB Post Doc Meeting
6:15 PM - 6:45 PM Conference 1

SRB Student Meeting
6:15 PM - 6:45 PM Conference 2

ESA AGM
6:15 PM - 7:15 PM James Hay

ESA / SRB Student Function
7:00 PM - 11:00 PM Annies

Tuesday, 4 September 2007

ESA Breakfast Workshop - Career Development for PhD Students and Post-Docs

8:15 AM - 9:45 AM — Limes Room

Chair: Helen MacLean

8:15am **Wayne Tilley**
How to write a successful grant *abs#119*

8:45am **Christine Clarke**
The importance of mentors *abs#120*

9:15am **Raymond Rodgers**
Getting Published *abs#121*

SRB MLA Plenary Lecture

8:30 AM - 9:30 AM — James Hay

Iain Clarke — **Sponsored by** mla MEAT & LIVESTOCK AUSTRALIA

GnRH and all the President's Men (a conspiracy theory) *abs#122*

ESA Clinical - Meet the Expert 2

8:30 AM - 9:30 AM — M3

Chair: Ken Ho

Annamaria Colao — **Sponsored by** IPSEN Innovation for patient care

GHD in adults and acromegaly *abs#123*

Morning Tea

9:30 AM - 10:00 AM — Exhibition Hall

SRB Orals 7 - Spermatogenesis and Sperm

10:00 AM - 12:00 PM — Conference 1

Chair: Sarah Meachem & Duangporn Jamsai

10:00am **Kiri Beilby**
Enhanced pregnancy rates following low dose insemination with sex-sorted ram sperm *abs#124*

10:10am **Vinali Dias**
Activin signalling modulators in normal, hormone-treated, and neoplastic human testis. *abs#125*

10:20am **Jennifer Ly-Huynh**
Specific nuclear targeting of chromatin remodelling factor, CDYL BY Importin α2 IS critical for its role in histone H4 hyperacetylation during spermatogenesis *abs#126*

10:30am **Sarah Meachem**
Gonadotropins regulate germ cell survival not proliferation, in normal adult men *abs#127*

10:40am **Stephanie Smith**
“Joey” – a novel model of male spermatogenic failure generated using ENU-mutagenesis *abs#128*

10:50am **Dean Whelan**
Identification of chaperone associated proteins potentially involved in gamete recognition and interaction. *abs#129*

11:00am **Paula Barlow**
Exposure to a high phytoestrogen diet from conception reduces Sertoli cell number in the 18 day old rat. *abs#130*

11:10am **Gerard Tarulli**
Stage-dependency of testicular tight junction localisation and expression. *abs#131*

11:20am **Zhen Zhang**
Bovine Sertoli cells survive and form tubular structure in and out of mouse testis *abs#132*

11:30am **Geoffry De Iuliis**
The impact of estrogenic compounds on DNA integrity in the male germ line *abs#133*

ESA Clinical - Orals and ESA Mayne Pharma Bryan Hudson Clinical Award

10:00 AM - 12:00 AM M3

Chairs: Elke-Christine Hendrich and Carolyn Allan
– the first three session presenters are Award Finalists

10:00am **Paul Lee**
Vitamin D as an analgesia for patients with type 2 diabetes and non-specific musculoskeletal pain - a prospective observational study. *abs#134*

10:15am **Bu Yeap**
Healthier lifestyle predicts higher subsequent serum testosterone and sex hormone binding globulin concentrations in older men. The Health In Men Study. *abs#135*

10:30am **Morton Burt**
Glucocorticoid-induced protein wasting: protein metabolic evaluation of the therapeutic potential of growth hormone and dehydroepiandrosterone *abs#136*

10:45am **Bernard Tuch**
Pilot clinical trial with human islets in barium alginate microcapsules *abs#137*

11:00am **Lisa Moran**
The use of Anti-Mullerian hormone in predicting menstrual response following weight loss in overweight women with Polycystic Ovary Syndrome *abs#138*

11:15am **Lyndal Tacon**
A case of dopamine beta hydroxylase deficiency causing severe autonomic neuropathy and responding to L-DOPS treatment *abs#139*

11:30am **Carolyn Allan**
Experience with Long Acting Intramuscular Testosterone Undecanoate *abs#140*

ESA / SRB Joint Orals - Female Reproduction Session

10:00 AM - 12:00 PM Limes Room

Chairs: Mai Sarraj and Darryl Russell

10:00am **Dave Grattan**
Ovarian steroids regulate SOCS expression in the hypothalamus: cross-talk between steroid and cytokine signal transduction in the brain. *abs#141*

10:15am **Kirsty Walters**
Reproductive Dysfunction in Female Androgen Receptor Null Mice ($AR^{-/-}$) Is Due to Extra-Ovarian Defects in Hypothalamic-Pituitary Regulation Rather than Intrinsic Ovarian Disorders *abs#142*

10:30am **Elizabeth Rivalland**
Is the suppression of luteinising hormone (LH) by cortisol in gonadectomised ewes affected by different levels of adiposity? *abs#143*

10:45am **Hannah Brown**
Hormonal control of ovarian blood and lymph vascular angiogenesis: Key processes in folliculogenesis and ovulation *abs#144*

11:00am **Theresa Hickey**
Androgen receptor-dependent stimulation of steroidogenesis in a human granulosa tumour cell line, KGN *abs#145*

11:15am **Lachlan Moldenhauer**
In vivo priming to paternal antigens is consistent with successful pregnancy outcome *abs#146*

11:30am **Janette Quennell**
Leptin action on neurons is required for normal puberty onset *abs#147*

11:45am **Cadence Minge**
Impaired oocyte developmental competence arises from diet-induced obesity and can be reversed by peri-ovulatory rosiglitazone treatment *abs#148*

ESA Orals - Metabolism & Obesity

10:00 AM - 12:00 PM James Hay

Chairs: Kathy Gatford, Brendan Waddell

10:00am **Belinda Henry**
Central leptin alters post-prandial thermogenesis in muscle and fat of the sheep *abs#149*

10:15am **Chen Chen**
Linoleic acid induces Ca^{2+}-dependent inactivation of L-type voltage-gated Ca^{2+} channels in primary cultured rat pancreatic beta-cells *abs#150*

10:30am **Brendan Waddell**
Programming of adult hypothalamic leptin resistance by fetal glucocorticoid excess in the rat *abs#151*

10:45am **Peter Mark**
Maternal dexamethasone treatment programs the adipocyte phenotype in adult offspring *abs#152*

11:00am **Lee Kennedy**
CXCL12 (stromal cell-derived factor-1) secretion by preadipocytes is enhanced by short-chain fatty acids (SCFAs), acting through a G protein-coupled receptor (GPR41) *abs#153*

11:15am **Michelle Van Sinderen**
The estrogenic component of tibolone reduces adiposity in female aromatase knockout (ArKO) mice - a model of menopause *abs#154*

11:30am **Miles De Blasio**
Effect of placental restriction on circulating leptin and its relationship to feeding activity and adiposity in the young lamb. *abs#155*

11:45am **Sue Mei Lau**
Increased adiposity in offspring of murine diabetic pregnancy is due to alterations in fuel metabolism. *abs#156*

ESA Orals - Growth Factors & Signalling

10:00 AM - 12:00 PM Conference 2

Chair: Vince Russo, Anne Nelson

10:00am **Heather Lee**
Oestrogen effects on prolactin induced STAT signalling *abs#157*

10:15am **Peter Leedman**
Regulation of growth factor receptor gene expression in human cancer by miRNAs *abs#158*

10:30am **Deborah Marsh**
Rapamycin treatment of a boy with Proteus syndrome and a germline *PTEN* mutation *abs#159*

10:45am **Sandra Higgins**
Anti-proliferative and pro-differentiation effects of FGF-2 on SK-N-MC cells involves regulation of id genes and inhibition of EMT-like mechanisms *abs#160*

11:00am **Anne Nelson**
The Influence of Gender and Testosterone on the Response to GH of IGF Axis and Collagen Markers in Young Recreational Athletes: a Double-blind Placebo-controlled Study. *abs#161*

11:15am **Graham Barrell**
effects of bovine somatotrophin on circulating IGF-1 concentration and milk production in red deer *abs#162*

11:30am **Warrick Inder**
dexamethasone administration inhibits skeletal muscle expression of the androgen receptor and IGF-1 – Implications for steroid-induced myopathy. *abs#163*

11:45am **Ken Ho**
Does Growth Hormone and Testosterone Supplementation Improve Physical Performance? A Double-blind Placebo-controlled Study in Recreational Athletes. *abs#164*

ESA Harrison Lecture

12:00 PM - 1:00 PM James Hay

Chair: Jeffrey Zajac

Gerard Karsenty
Endocrine Regulation of Energy Metabolism by the Skeleton *abs#165*

SRB Lunch Including Student Lunch with the Founder's Lecturer

12:00 PM - 1:00 PM Conference 1

ESA Lunch

1:00 PM - 2:00 PM Exhibition Hall

Sponsored by NOVARTIS ONCOLOGY

SRB Orals 8 - Uterine function in health and disease

1:00 PM - 3:00 PM Conference 1

Chair: Louise Hull & Lois Salamonsen

1:00pm **Tu'uhevaha Kaitu'u-Lino**
Estrogen is not essential for full endometrial restoration in a mouse model. *abs#169*

1:10pm **Jim Peterson**
Uterine gene expression differences between superior and inferior recipient cows. *abs#170*

1:20pm **Jeffrey Keelan**
Lysophospholipids May Influence the Onset of Labour by Increasing Expression of Contractile Associated Proteins in Human Myometrium *abs#171*

1:30pm **Lynette Kilpatrick**
Proteomic Identification of Proprotein Convertase 6 Substrates in Decidualised Human Endometrial Stromal Cells *abs#172*

1:40pm **Mary Hull**
MicroRNA expression in Endometriosis *abs#173*

1:50pm **Naomi Morison**
Mifepristone treatment halts Implanon-associated breakthrough bleeding by enhancing endometrial repair. *abs#174*

2:00pm **Laura Lindsay**
A switch from paracellular to transcellular fluid transport mechanisms in rat uterine epithelial cells at the time of implantation *abs#175*

2:10pm **Chelsea Stoikos**
BMP-2 and TGFβ-1 increase during human endometrial stromal cell decidualization *abs#176*

2:20pm **Laura Venuto**
Apical distribution of EZRIN in all rat uterine epithelial cells at the time of implantation except those within the implantation chamber *abs#177*

2:30pm **Jane Girling**
Oestrogen has differential effects on VEGF-A isoform and receptor mRNA expression in different cellular compartments of the mouse uterus *abs#178*

SRB Symposium - Evolution of Reproductive Form and Function. Novel Models, Novel Insights

1:00 PM - 3:00 PM Limes Room

Chair: Jane Girling

1:00pm **Susan Jones**
Hormonal control of gestation and parturition in viviparous lizards *abs#166*

1:30pm **Gary Hime**
Genetics of stem cells – how fruitflies can assist reproductive technology *abs#167*

2:00pm **Alex Quinn**
Sex in dragons: A theoretical framework for the evolution of sex determination in reptiles. *abs#168*

ESA Clinical - Meet the Expert 3

1:30 PM - 2:30 PM Conference 2

Chair: Peter Ebeling

Ian Reid
Osteoporosis Management *abs#179*

ESA Tuesday Poster Session

2:00 PM - 3:00 PM Upstairs Foyer

See listing at end of program.

Even numbered abstracts to be attended

Topics: Development and Immunology; Cancer; Technical; Growth Hormone; Nuclear Hormone Receptors; Pituitary; Clinical II

ESA / Neuroendocrinology Australasia Joint Symposium - Gut/brain Axis

3:00 PM - 5:00 PM James Hay

Chair: Brian Oldfield and Belinda Henry **Sponsored by** Lilly Diabetes Your journey inspires ours.

3:00pm **Matthias Tschoep**
The role of Ghrelin and its CNS targets in the control of energy metabolism *abs#180*

3:30pm **Andrew Young**
Amylinomimetics for Metabolic Diseases *abs#181*

4:00pm **Anthony Verberne**
New tricks for an old gut peptide: cholecystokinin and sympathetic outflow to the splanchnic circulation *abs#182*

4:30pm **Herbert Herzog**
The Role of PYY in regulating energy balance and glucose homeostasis *abs#183*

ESA / AACB Joint Clinical Symposium - Endocrinology: The Clinic-Laboratory Interface

3:00 PM - 5:00 PM Limes Room

Chair: Chris Florkowski & Cherie Chiang **Sponsored by** novo nordisk®

3:00pm **Bruce Robinson**
Gene testing – its role in endocrinology*abs#184*

3:30pm **John Lainchbury**
Natriuretic Peptides and Adjustment of Therapies in Cardiac Failure *abs#185*

4:00pm **John Lewis**
Salivary steroids *abs#186*

4:30pm **Robert Baxter**
Insulin-like growth factors and their binding proteins in endocrinology and cancer *abs#187*

ESA Orals - Pregnancy, Prostate & Reproduction

3:00 PM - 5:00 PM Conference 1

Chairs: Theresa Hickey and Vicki Clifton

3:00pm **Birunthi Niranjan**
Estrogen dendrimer conjugates activate nongenomic pathways of estrogen action in prostate cells *abs#188*

3:15pm **Margaret Morris**
Maternal obesity in the rat leads to increased body weight, adiposity, insulin and impaired glucose tolerance in offspring *abs#189*

3:30pm **Ulla Simanainen**
Androgen responsiveness of anterior and dorsolateral prostate in prostate epithelial-specific androgen receptor knockout (PEARKO) mice *abs#190*

3:45pm **Naomi Scott**
Placental inflammatory response in pregnancies complicated by asthma *abs#191*

4:00pm **Kathryn Gatford**
Placental and fetal growth restriction reduce β-cell mass before and after birth in the sheep *abs#192*

4:15pm **Dina Zebian**
Human *In vitro* Fertilisation (IVF) derived granulosa cells: *in vitro* characterisation and comparison of two viability assays *abs#193*

4:30pm **Ellen Podivinsky**
Manhood Threatened: Could Estrogenic Pesticides Affect Male Fertility? *abs#194*

4:45pm **Kristy Shipman**
Investigation into the Effect of CREAP on CRH Promoter Activity in JEG-3 Cells *abs#195*

ESA Orals - Thyroid & Parathyroid

3:00 PM - 5:00 PM Conference 2

Chairs: John Walsh and Penny Hunt

3:00pm **Suzanne Brown**
Heritability of serum TSH, free T4 and free T3 concentrations: A study of a large UK twin cohort *abs#196*

3:15pm **Rhonda Gilbert**
First trimester specific reference intervals for thyroid hormones *abs#197*

3:30pm **Ash Gargya**
Trimester-specific thyroid function test reference ranges for iodine sufficient pregnant women attending an ambulatory antenatal clinic *abs#198*

3:45pm **Deborah Marsh**
Nuclear and nucleolar localisation of parafibromin, the putative tumour suppressor associated with Hyperparathyroidism Jaw Tumour syndrome and sporadic parathyroid carcinoma *abs#199*

4:00pm **Rosemary Wong**
rhTSH (Thyrogen®) in thyroid cancer follow up: experience at a single institution *abs#200*

4:15pm **Ashley Makepeace**
Significant association between serum free thyroxine concentration and body mass index in euthyroid subjects: a community-based study. *abs#201*

4:30pm **Mathis Grossmann**
Thyrotoxicosis during Sunitinib Treatment for Renal Cell carcinoma *abs#202*

4:45pm **Ravi Gayathri**
Prevalence of subclinical hypothyroidism and autoimmune thyroiditis in pregnancy *abs#203*

SRB Afternoon Tea

3:00 PM - 3:30 PM Exhibition Hall

SRB Young Investigators

3:30 PM - 5:00 PM Cambridge

Chair: Michael Holland **Sponsored by** serono biotech & beyond

3:30pm **Cathryn Hogarth**
Nuclear transport at the onset of mammalian gonad sexual differentiation: Identification of cargo for importin alpha 2 in the fetal testis. *abs#204*

3:45pm **Deirdre Zander-Fox**
Embryo programming: The involvement of mitochondria *abs#209*

4:00pm **Gayathri Rajaraman**
Homeobox gene *HLX1* is a mediator of HGF-stimulated trophoblast migration *abs#207*

4:15pm **Ambika Singh**
Studies of the role of ABC transporters and sphingolipids in trophoblast differentiation *abs#208*

4:30pm **Rachael Nowak**
Paternal polymorphisms are associated with pregnancies complicated with uteroplacental insufficiency *abs#206*

4:45pm **Georgia Kafer**
Evidence for a glucose sensing system regulating physiology of the preimplantation mouse embryo *abs#205*

SRB AGM

5:00 PM - 6:00 PM Cambridge

ESA / NZSE Joint Plenary – NZSE Nancy Sirrett Lecture

5:15 PM - 6:15 PM James Hay

Chair: Dave Grattan

Jeff Keelan, Placental mediators of pregnancy and parturition *abs#210*

ESA / SRB Conference Dinner

7:30 PM - 11:00 PM Limes Room

Sponsored by IPSEN Innovation for patient care

ESA Japan-Australia / ADS Joint Plenary Lecture

8:30 AM - 9:30 AM James Hay

Chair: Helen MacLean & Terri Allen

Takashi Kadowaki

Adiponectin and its receptors in insulin resistance, diabetes, and metabolic syndrome, and obesity *abs#211*

SRB Orals 9 - Male reproductive tract development and function

8:30 AM - 9:30 AM Cambridge

Chair: Moira O'Bryan & Jenny Ly-Hunh

8:30am **Mai Sarraj**
The murine betaglycan gene is essential for normal seminiferous cord formation. *abs#212*

8:40am **Mark Hedger**
Production of regulatory cytokines by lymphocyte subsets in the rat testis is consistent with its status as an immune privileged site *abs#213*

8:50am **Melissa Gamat**
SHBG and megalin expression in the developing reproductive tract of a marsupial, the tammar wallaby *abs#214*

9:00am **Helen Gehring**
The expression of steroid 5α-reductases in reproductive tissues during virilisation in the tammar wallaby, *Macropus Eugenii*. *abs#215*

9:10am **Sirisha Mendis**
Activin-regulated genes in the developing mouse testis *abs#216*

9:20am **Trina Jorre de St Jorre**
Selection of rams for temperament does not affect their reproductive capacity *abs#217*

SRB Orals 10 - Germ cells and Stem cells

8:30 AM - 9:30 AM M4 & M5

Chair: Michelle Lane and Julia Young

8:30am **C Anees**
Development of totipotent stem cell line and their characterization *abs#218*

8:40am **Caroline Gargett**
Role of Epithelial Stem/Progenitor Cells in Estrogen-induced Endometrial Regeneration *abs#219*

8:50am **Peter Kaye**
IGF-I and insulin activate mitogen activated protein kinase via the type 1 IGF receptor in mouse embryonic stem cells *abs#220*

9:00am **Shaun Roman**
Isolating and genotyping spermatogonial stem cells *abs#221*

9:10am **Julia Young**
Embryonic stem cells: an approach to germline genesis *abs#222*

Morning Tea

9:30 AM - 10:00 AM James Hay

ESA / ADS Joint Basic Symposium - Recent Advances in Insulin Signal Transduction

10:00 AM - 12:00 PM James Hay

Chair: Mark Febbraio & John Whitehead

10:00am **Colin Ward**
Structural insights into ligand-induced activation of the insulin receptor *abs#223*

10:20am **Ronald Kahn**
Critical nodes in signalling pathways: insights into insulin action *abs#224*

10:50am **David James**
Dissecting multiple steps of GLUT4 trafficking and identifying the sites of insulin action *abs#225*

11:20am **Takashi Kadowaki**
New roles of IRS-2 in compensatory beta cell hyperplasia and vascular function *abs#226*

ESA Basic Symposium – TGFβ Superfamily in Development & Disease

10:00 AM - 12:00 PM M4 & M5

Chairs: Jean Flemming and Kaye Stenvers

10:00am **Ian McLennan**
The gonadal hormone, Müllerian Inhibiting Substance, has cryptic functions in the central nervous system. *abs#227*

10:30am **Martin Matzuk**
TGFβ Superfamily Signaling and Gonadal Function *abs#228*

11:00am **David Phillips**
The new endocrinology of activin and follistatin: exploring their roles in inflammation and other critical diseases *abs#229*

11:30am **Wendy Ingman**
The essential roles of TGFβ1 in reproductive biology *abs#230*

ESA Clinical Symposium - Recent Advances in Thyroid Disease and Management

10:00 AM - 12:00 PM M6 & M7

Chairs: Rosemary Wong and John Burgess

10:00am **Robin Mortimer**
Thyroid Cancer -Issues *abs#235*

10:30am **Penny Hunt**
Genetics of Autoimmune Thyroid Disease *abs#236*

11:00am **John Walsh**
Thyroid hormone replacement *abs#237*

11:30am **Jonathan Serpell**
Thyroid surgery *abs#238*

ESA / SRB Joint Basic Symposium - Endocrine Disruptors

10:00 AM - 12:00 AM Conference 1

Chairs: Iain Clarke and Darryl Russell

10:00am **Louis Guillette**
Endocrine disruption and the development of the reproductive system: Lessons from wildlife *abs#231*

10:30am **Steve Assinder**
A high phytoestrogen diet disrupts male reproductive function. *abs#232*

11:00am **Shaun Roman**
Expression and activity of Phase I detoxifying enzymes in the male germ line of the mouse. *abs#233*

11:30am **Gail Risbridger**
Transient endocrine disruption induces prostate pathologies upon aging. *abs#234*

SRB Orals 11 - Oocytes and Embryos

10:00 AM - 12:00 PM Conference 2

Chair: Ken McNatty and Chris Grupe

10:00am **Michelle Lane**
Media for pre-implantation embryo culture alters the quality of resultant ICM colonies in the mouse *abs#239*

10:10am **Jiang-Hua Shang**
Production of rabbit blastocysts by nuclear transfer of adult bone marrow mesenchymal stem

cells *abs#240*

10:20am **Kylie Dunning**
Functional characterisation of the cumulus oocyte matrix during maturation of oocytes *abs#241*

10:30am **Megan Mitchell**
Epigenetic changes in the murine oocyte and embryo following maternal dietary protein supplementation *abs#242*

10:40am **Christopher Grupen**
Production of monozygotic twin lambs from sex-sorted sperm by embryo bisection *abs#243*

10:50am **Christine Yeo**
Disruption of bi-directional oocyte-cumulus paracrine signalling during in vitro maturation reduces subsequent mouse oocyte developmental competence *abs#244*

11:00am **Sara Edwards**
The co-operative effect of GDF9 and BMP15 on granulosa cell function is modulated primarily through BMP Receptor II *abs#245*

11:10am **Kelly Banwell**
Mitochondrial consequences following *in vitro* maturation (IVM) of mouse oocytes in varying oxygen concentrations. *abs#246*

11:20am **Karen Reader**
A quantitative ultrastructural study of early stage oocytes from wild-type and booroola sheep *abs#247*

ESA Clinical Lunchtime Workshop - Debate: That Measuring Serum Insulin as a Marker of Insulin Resistance has Clinical and Diagnostic Utility

12:00 PM - 1:00 PM M6 & M7

Chairs: Frank Alford

12:00pm **Warren Kidson**
Insulin resistance testing – clinical and community utility *abs#248*

12:30pm **Helena Teede**
Measuring serum insulin as a marker of insulin resistance does not have clinical and diagnostic utility. *abs#249*

Lunch

12:00 PM - 1:00 PM Exhibition Hall

SRB Symposium - Reproductive Biotechnology in Animal Industry

1:00 PM - 3:00 PM Cambridge

Chair: Shaun Roman **Sponsored by** CENTRE FOR REPRODUCTION AND GENOMICS

1:00pm **Mark Nottle**
Biomedical and agricultural applications of porcine cloning *abs#250*

1:30pm **David Wells**
Nuclear transfer in cattle: a reproductive tool to generate animals from an existing or modified genome. *abs#251*

2:00pm **Stefan Hiendleder Hiendleder**
Nuclear-mitochondrial interactions in cloned animals *abs#252*

2:30pm **Paul Verma**
Bovine embryonic stem cells: isolation, characterization and potential applications *abs#253*

ESA POSTERS

Odd-numbered abstracts presented Monday 12:00-1:00 pm, even-numbered abstracts presented Tuesday 2:00-3:00 pm

Jorge Tolosa
Immunosuppressive properties of a synthetic peptide analogous to human Syncytin immunosuppressive peptide *abs#254*

David Phillips
Preliminary definition of the mechanisms by which activin A is released from cells and its response to an inflammatory stimulus *abs#255*

Hui Kheng Chua
Expression of the Murine Betaglycan Gene in the Foetal Leydig Cells during Gonadogenesis in the Mouse. *abs#256*

Jeremy Smith
Increased luteinising hormone response to kisspeptin during the anestrous season in ewes *abs#257*

Patricia Grant
Pregnancy and nutrition differentially regulate expression of IGF-I and IGF-II in the Guinea pig *abs#258*

John Schjenken
Potential Role of CRH in Mediating Immune Function in Pregnancy *abs#259*

Nicolette Hodyl
Pre-eclampsia affects incidence of infants born small for gestational age differentially depending on both infant sex and degree of prematurity *abs#260*

Nicolette Hodyl
A developmental profile of the capacity of the innate immune system to respond to lps following prenatal endotoxin exposure in the rat *abs#261*

Isabelle Hoong
Molecular cloning and characterization of bovine corticosteroid binding globulin during fetal development. *abs#262*

Inga Mertens
Protein kinase C mediates gonadotropin induced mitogen-activated protein kinase signalling in epithelial ovarian cancer cell lines *abs#264*

Kym Rae
Follistatin, Activin A and other Inflammatory proteins through Parturition *abs#265*

Andrew Sakko
Phosphorylated androgen receptor levels as a biomarker in early stage prostate cancer *abs#266*

Kathryn Woad
Investigating the association between *INHA* promoter polymorphisms and premature ovarian failure. *abs#267*

Takafumi Taguchi
The role of transcriptional co-factors in the regulation of PAX8-PPAR γ in thyroid cells *abs#268*

Kathryn Backholer
Kisspeptin cells project to pro-opiomelanocortin (POMC) and neuropeptide Y (NPY) cells in the arcuate nucleus of the ewe; evidence for transmission of sex steroid feedback to appetite regulating cells. *abs#269*

Jennifer Wong
Lessons from a Review of Thyroglobulin Assays in the Management of Thyroid Cancer *abs#270*

G. Almahbobi
The follicle size and flushing determine the rate of oocyte retrieval *abs#271*

Javed Iqbal
Rapid *in vivo* Effects of Estrogen in Ovine Pituitary Gonadotropes, leading to phosphorylation of ERK and CREB *abs#272*

John Schjenken
An Improved Method to Simultaneously Extract DNA, RNA and Protein from the same Sample *abs#273*

Deborah Prendergast
Mutational analysis of the SPRASA gene in infertile couples *abs#274*

Liping Chung
Proteomic Classification of the Human White Blood Cell Response to Growth Hormone by Protein Expression Profiling *abs#275*

Kati Matthiesson
A randomised four-way cross over study to compare the steady-state pharmacokinetics of testosterone following application of different Testosterone Metered Dose (MD) Lotion® formulations and doses and Androgel® in healthy male volunteers with suppressed t *abs#276*

Larissa Christophidis
Role for Growth Hormone in Neuro-restoration Subsequent to Focal Ischemia in the Immature Rat Brain. *abs#277*

Vita Birzniece
Testosterone Stimulates Extra-Hepatic but not Hepatic Fat Oxidation at Systemic Replacement Doses in Hypopituitary Men *abs#278*

Vita Birzniece
Growth Hormone and Testosterone Exert Differential and Additive Effects on Lean Body Mass in Recreational Athletes *abs#279*

A Setiawan
Effects of 11-ketotestosterone on hepatic physiology of the shortfinned eel, *Anguilla australis*, *in vivo*. *abs#280*

Shu-Ching Wang
ERRgamma, an orphan NR regulator of metabolic gene expression in skeletal muscle cells. *abs#281*

Kun Wang
Adiponectin elevates $[Ca^{2+}]i$ in rat somatotropes through activation of adiponectin receptors leading to growth hormone secretion in vitro *abs#282*

Jyotsna Pippal
Characterisation of the n/c-interaction in the mineralocorticoid receptor *abs#283*

Melanie Tran
Shiftwork simulation in rats impairs glucose tolerance and insulin secretion and sensitivity *abs#284*

Nimalie Perera
The Role of Inferior Petrosal Sinus Sampling for ACTH- dependent Cushing's syndrome. *abs#285*

Yufeng Zhao
Linoleic acid induces an increase in intracellular calcium concentration and membrane hyperpolarization of primary cultured rat pancreatic β-cells *abs#286*

Mark McLean
Efficacy and safety of Lanreotide Autogel in patients with acromegaly previously treated with Octreotide LAR. *abs#287*

Sue-Lynn Lau
Infectious causes of of adrenal insufficiency - prevalence, clinical features and long term follow-up in a South Indian tertiary hospital. *abs#288*

Ivan Kuo
Lugol's iodine in preparation for thyroidectomy *abs#289*

Nicholas Kasmeridis
Large secretory ganglioneuroma in a 19 year old man. *abs#290*

Stephanie Maclean
Annual zoledronic acid as anti-resorptive therapy for osteoporosis in clinical practice *abs#291*

Walter Plehwe
Urinary Iodine Concentrations in Early Pregnancy. *abs#292*

Winnie Ho
A case of hypercalcemia, adrenal insufficiency and thyrotoxicosis *abs#293*

Ann McCormack
Measurement of Glucagon-Like Peptide 1 (GLP-1) levels in Noninsulinoma Pancreatogenous Hypoglycaemia Syndrome (NIPHS) *abs#294*

Kingsley Nirmalaraj
An Audit of Treatment Outcomes of Graves' Disease and Toxic Multinodular Goitre in South Auckland. *abs#295*

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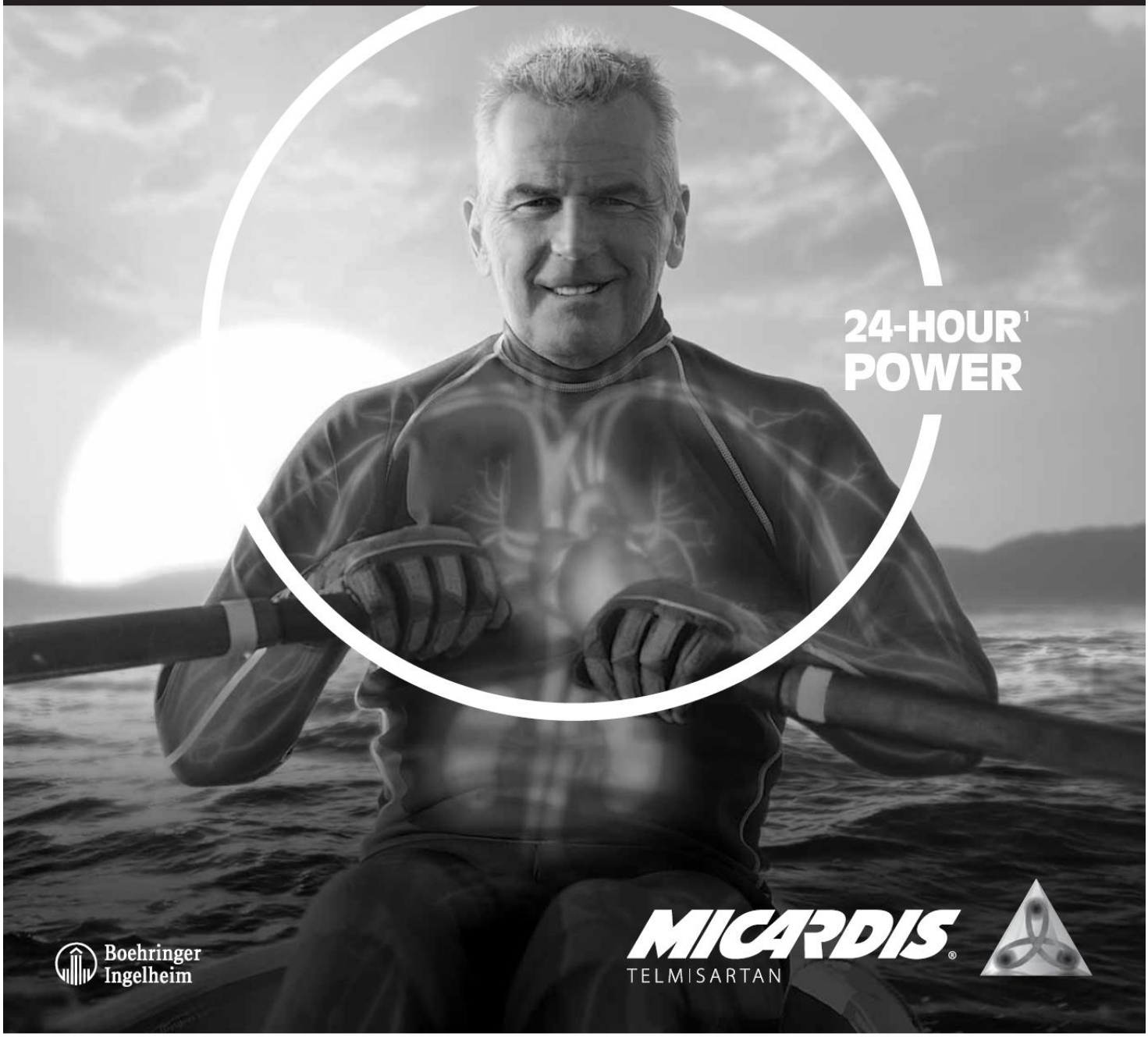

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Ph: 02 9594 5550
www.pitutiary.asn.au
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www.cslbiotherapies.com.au
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Ph: 02 6232 3800
www.diabetesaustralia.com.au
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Diabetes Australia provides practical assistance and information to people with all types of diabetes across Australia. On behalf of the Australian Government, Diabetes Australia also administers the National Diabetes Services Scheme (NDSS) which provides subsidised products to over 800,000 people with diabetes.

Diagnostic Systems Laboratories Australia Pty Ltd **Site 34**
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Endocrine Society of Australia (ESA) **Site 51**
SYDNEY NSW 2000
Ph: 02 9256 5405
www.racp.edu.au/esa
The Endocrine Society of Australia is a national non-profit organisation of scientists and clinicians who conduct research and practice in the field of Endocrinology. The society was founded in 1958 and incorporated in 1986 in the State of Victoria. The Society is governed by the 8 members of its Council who are elected every two years by a ballot of the membership in accordance with the Constitution. Visit the site to meet the Endocrine Society secretariat.

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(1) Janka H, et al Diabetes Care 2005; 28: 254-259 (2) Riddle M, et al Diabetes Care 2003; 26(11):3080-3086 (3) Raskin P et al Diabetes Care 2005; 28(2):260-265 (4) IMS WW Volume Quarter 1, 2007.

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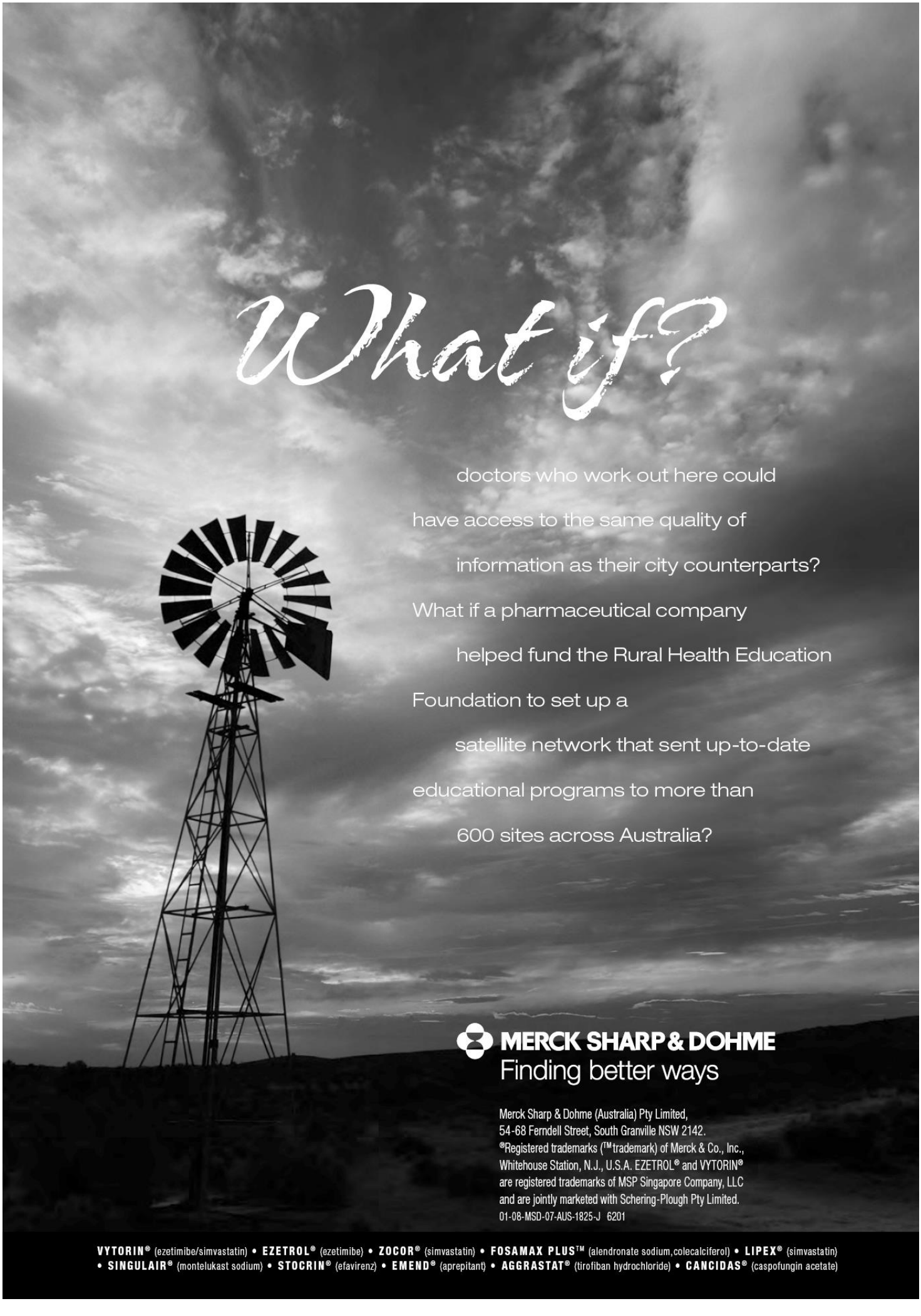
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AUTHOR INDEX

ORALS

001

SCIENTIFIC JOURNAL PUBLISHING

S. T. Mortimer

CSIRO Publishing, Collingwood, VIC, Australia

While publishing experimental results is a critical part of the research process, preparing papers is often seen as a daunting task, particularly by new investigators. The aim of this Workshop is to demystify some of the aspects of publishing, and to provide some tools for the preparation and submission of manuscripts. Three areas of publishing will be covered:

- the publication process - where we are now, and where we are going in the future
- preparing and submitting papers - hints and tips about what needs to be included, and what the referees (and readers) expect
- manuscript reviewing - how to provide a meaningful critical review

002

OUT OF CONTROL: THE TRANSFORMING GROWTH FACTOR-B SUPERFAMILY IN EARLY SPERMATOGENESIS AND ITS POTENTIAL RELEVANCE TO TESTICULAR DYSGENESIS

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Regulated transforming growth factor-β (TGFβ) superfamily signaling is required throughout testicular development and for full spermatogenesis. Because many members of this family signal through shared receptors, signal transduction components and inhibitors, the ligands in this family exhibit functional overlaps; depending on the specific cellular context, these ligands may compete with or compensate for each other. Our studies of testis development in rodents have implicated the TGF-β superfamily ligand activin and its inhibitors in the modulation of both germ cell and Sertoli cell development. Stereological analyses of 3 genetically distinct mouse models have demonstrated a direct association between establishment of germ cell numbers in the fetal testis and altered levels of bioactive activin. Sertoli cell proliferation is also affected, and in vivo and in vitro analyses are underway to identify the relevant signalling pathways and their biochemical and physiological outcomes at several ages of development. Examining this signalling pathway in the adult human testis has led us to discover that synthesis of one particular activin receptor subunit is altered in spermatogonia and Sertoli cells in association with changes in both circulating hormones and in the progression to form the seminomas of testicular cancer. These outcomes highlight the need to better understand the implications of altered activin signalling for actions of other TGF-β ligands and how this is acheived.

003

GENETIC VARIANTS IN GDF9 AND BMP15 IN MOTHERS OF DIZYGOTIC TWINS

G. W. Montgomery[1], Z. Z. Zhao[1], J. S. Palmer[1], N. G. Martin[1], D. I. Boomsma[2], D. L. Duffy[1]

[1]Molecular Epidemiology, Queensland Institute of Medical Research, Brisbane, QLD, Australia
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Genetic factors contribute to an increased chance of having dizygotic (DZ) twins. The ovarian bone morphogenetic signalling pathway genes (GDF9 and BMP15) are critical for normal human fertility and implicated in twinning variation. We analysed common variants in both GDF9 and BMP15 in families with a history of DZ twinning and also screened for rare variants in GDF9 and BMP15 in 279 unrelated mothers of DZ twins. Rare variants identified by denaturing high performance liquid chromatography (DHPLC) were confirmed by DNA sequencing. Both common and rare variants were typed by MALDI-TOF mass spectrometry in our DZ twinning families. Common variants in either GDF9 or BMP15 were not significantly associated with DZ twinning. We found two novel insertion/deletions (c.392-393insT, c.1268-1269delAA) and four missense alterations (p.Pro103Ser, p.Thr121Leu in the pro-region and p.Pro374Leu and p.Arg454Cys in the mature protein region) in the GDF9 sequence in mothers of DZ twins. The frequency of all GDF9 variants was significantly higher ($P<0.0001$) in mothers of twins (4.12%) compared with controls (2.29%). In contrast, we identified four missense variants (p.Pro174Ser, p.Phe194Ser, p.Ala311Thr, pArg391Thr) in BMP15 in mothers of DZ twins, but there was no evidence for an increased frequency of these rare variants. We conclude that rare variants in GDF9, but not BMP15 are associated with DZ twinning. However, these variants account for only a small part of twinning variation.

004

OOCYTE-SECRETED FACTOR REGULATION OF FOLLICLE AND OOCYTE MATURATION.

R. B. Gilchrist

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

Oocyte quality is a key limiting factor in female fertility and in artificial reproductive technologies, yet we have a poor understanding of what constitutes oocyte quality or the mechanisms governing it. The ovarian follicular environment and maternal signals, mediated primarily through granulosa cells and cumulus cells (CCs), are responsible for nurturing oocyte growth, development, and the gradual acquisition of oocyte developmental competence. However, oocyte-CC communication is bidirectional, and a new concept that is emerging, which will be the focus of this presentation, is that the oocyte secretes potent growth factors that act locally to direct the differentiation and function of CCs. Two important oocyte-secreted factors (OSFs) are growth differentiation factor 9 and bone morphogenetic protein 15, which activate known signalling pathways in CCs to regulate key genes and cellular processes required for CC differentiation and for CCs to maintain their distinctive phenotype. Hence, oocytes appear to tightly control their neighboring somatic cells, directing them to perform functions required for appropriate development of the oocyte. This oocyte-CC regulatory loop and the capacity of oocytes to regulate their own microenvironment by OSFs, may constitute important components of oocyte quality. In support of this notion, we have recently demonstrated that supplementing oocyte in vitro maturation (IVM) media with exogenous OSFs improves oocyte developmental potential, as evidenced by enhanced pre- and post-implantation embryo development. This new perspective on oocyte-CC interactions is improving our knowledge of the processes regulating oocyte quality, which is likely to have a number of applications, including improving the efficiency of clinical oocyte IVM and thereby providing new options for the treatment of infertility.

005

UNDER ATTACK: GERM CELL DEFENCES AGAINST ENVIRONMENTAL TOXINS?

E. A. McLaughlin

Biological Sciences, University of Newcastle, Callaghan, NSW, Australia

All female mammalian ovaries contain a limited supply of primordial follicles which are present from birth. Xenobiotics, such as organochlorine pesticides, polychlorinated biphenyls, dioxins, phthalates and synthetic oestrogens are capable of interfering with normal female reproductive function. Some xenobiotics prevalent in the environment including 4-vinylcyclohexane (VCD) have been shown to target primordial follicles and trigger atretic oocyte depletion of the ovary leading to premature menopause. Mammalian cells have three defence mechanisms for the elimination of xenobiotics. However bioactivation of xenobiotics by the Phase I enzymes (cytochrome P450s or Cyp's) in cells may have undesirable consequences such as the generation of free oxygen radicals (ROS) and subsequent DNA damage.

Our microarray screens determined that a number of Cyp genes including xenobiotic metabolising enzymes Cyp1b1 and Cyp2e1 were expressed at high levels in neonatal and adult mouse ovary. Using qPCR, we confirmed that exposure of neonatal ovaries to VCD in our *in vitro* culture system, resulted in upregulation of these enzymes in response to toxic attack. Additionally immunohistochemistry confirmed expression of Cyp1b1 and Cyp2e1 in the oocyte and granulosa cells of the murine ovary. Cumulus cell free oocytes were incubated in xenobiotics in supportive oocyte culture media. ROS production in oocytes was detected with Dihydroethidium (DHE) and cell vitality confirmed by exclusion of nuclear stain SYTOX® Green, using confocal microscopy. Colocalisation studies with mitochondrial probe Mitotracker Green® indicated that ROS production was largely confined to the mitochondria in xenobiotic treated oocytes. Using our *in vitro* culture system, we assessed the effects of direct exposure to the xenobiotic VCD on folliculogenesis. Short term treatment with VCD resulted not in only in apoptotic oocyte death but in premature activation of quiescent primordial follicles compared with control ovaries. This surprising result led us to suspect that intracellular signalling pathways were being perturbed by ROS production in the primordial follicles. Accordingly we assessed the gene and protein expression of p63, a known regulator of spontaneous apoptosis in murine oocytes. qPCR revealed substantial increase in p63 gene expression in treated ovaries and upregulation of p63 was corroborated by immunohistochemical and immunoblotting studies in oocytes of VCD treated ovaries.

In summary our recent studies of the effects of xenobiotic exposure in the murine ovary have demonstrated that environmental agents can cause significant primordial follicle loss and oocyte damage through oxidative stress. With Australian women opting to delay childbirth, life long exposure of ovarian oocytes to xenobiotics has repercussions both for the fertility of these women and the welfare of their offspring.

006

A MOLECULAR JOURNEY FROM INSULIN AND IGFS TO NEURODEGENERATIVE DISEASE

R. J. Smith

Division of Endocrinology, Rhode Island Hospital, The Warren Alpert Medical School of Brown University, Providence, RI, United States

Insulin and the insulin-like growth factors (IGF-I and IGF-II) have important functions in metabolic regulation and growth. Inherited mutations and acquired abnormalities in insulin and IGF signaling proteins contribute to diabetes mellitus and growth disorders. In addition to their global physiological functions, insulin and the IGFs have actions in specific tissues that may contribute to other

human diseases. There recently has been substantial interest in insulin and IGF effects in the central nervous system and the role of altered insulin and IGF signaling as potential causes of neurodegenerative disease. Signaling by insulin and the IGFs occurs through activation of an intrinsic receptor tyrosine kinase, and their cellular actions depend importantly but not exclusively on protein tyrosine phosphorylation. To investigate the role of phosphotyrosine-independent mechanisms in mediating or regulating insulin and IGF signaling, we used interactive cloning methods to search for proteins that bind to the intracellular portion of insulin and IGF receptors. This resulted in the identification of Grb10 as a protein that is recruited to activated insulin and IGF receptors. Studies by our group and others have shown that Grb10 negatively regulates tyrosine phosphorylation-based insulin and IGF signaling. Since Grb10 has the structure of an adapter protein, we further investigated its cellular function using interactive cloning to search for proteins that bind to Grb10 and thus may be recruited to insulin and IGF receptors by Grb10. One of the novel proteins that we identified with this method is GIGYF2 (Grb10-Interacting GYF Domain Protein 2). As an approach to studying GIGYF2 function, we examined human genome data and found strong linkage of the GIGYF2 locus on chromosome 2 to familial Parkinson's disease. This is of interest, since recently published findings have shown a nearly 2-fold increased incidence of Parkinson's disease in patients with type 2 diabetes. We sequenced the coding region of the GIGYF2 gene in 123 Italian and 126 French patients with familial Parkinson's disease and identified 10 different mutations (present in 6% of patients and absent from controls). We next generated mice with disruption of the GIGYF2 gene. GIGYF2 normally is abundantly expressed in both developing and adult mouse brain. The homozygous (-/-) knockout mice die shortly after birth, illustrating critical function(s) for GIGYF2. Heterozygous (+/-) GIGYF2 mice survive and have normal postnatal growth and blood glucose levels. However, at 12 months of age, the GIGYF2 (+/-) mice develop motor ataxia associated with degeneration of motor neurons, inclusion body-like structures, and alpha-synuclein-positive neuritic plaques in the spinal cord, brain stem, and cerebral cortex. These data strongly support a role for GIGYF2 as a novel human Parkinson's disease gene and suggest that the identified mutations result in loss of function of GIGYF2. Further studies on GIGYF2 may provide insight into the mechanistic basis of insulin and IGF effects in the central nervous system and their implications for neurodegenerative disease.

007

DEVELOPMENTAL PROGRAMMING: EFFECTS OF PRENATAL TESTOSTERONE EXCESS ON FOLLICULAR RECRUITMENT AND DEPLETION

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An array of adult reproductive disorders that include LH excess, functional hyperandrogenism, neuroendocrine defects, multifollicular ovarian morphology, and corpus luteum dysfunction culminating in early reproductive failure, is found in prenatal testosterone (T) treated sheep, the reproductive and metabolic phenotype of which mimic women with polycystic ovary syndrome. Multifollicular ovarian morphology originates from enhanced follicular recruitment and follicular persistence. We tested if enhanced follicular recruitment was programmed by androgenic actions of T and if it resulted in early depletion of follicular reserve. Pregnant sheep were given twice weekly injections (im)of 100 mg T propionate or dihydrotestosterone (DHT), a non-aromatizable androgen, from days 30 to 90 of gestation. Ovaries were obtained from day 90 and 140 fetuses and from 9-month old females during a synchronized follicular phase (n =6-9 per treatment). Stereological techniques were used to quantify changes in ovarian follicle/germ cell populations. There were no differences in number of oocytes and follicles between control (C), prenatal T- and DHT- treated animals on fetal day 90. Greater number of early growing follicles (primary) were found in prenatal T- but not DHT-treated fetuses on fetal day 140 compared to C (T: 3848 +/- 680, C: 1521 +/-25; $p<0.05$). Increased number growing follicles (primary to antral) and reduced number of primordial follicles were found in 9-month old prenatal T-treated females compared to C females (growing follicles, T: 251 +/- 17 vs. C: 159 +/-34; $P <0.05$; primordial follicles, T: 23167 +/-3042 vs. C: 56832 +/-7989, $P<0.05$). Prenatal DHT-treated females did not differ from C females. Ovaries of prenatal T-treated females also manifested additional morphological abnormalities, which included the appearance of numerous haemorrhagic follicles, abnormal early antrum formation and differences in the mesonephric remnant located in the medullary region of fetal day 140 ovary. These findings provide evidence that prenatal T excess leads to early follicular depletion and this programming is not due to androgenic actions of T.

NIH P01 HD44232

008

DEVELOPMENTAL COMPETENCE OF OOCYTES RELATES TO OVARIAN FOLLICULAR BASAL LAMINA PHENOTYPE

H. F. Irving-Rodgers[1], S. Morris[1], R. A. Collett[1], T. T. Peura[1], J. G. Thompson[1], M. Davy[2], H. D. Mason[3], R. J. Rodgers[1]

[1]Research Centre for Reproductive Health / Obstetrics and Gynaecology, University of Adelaide, Adelaide, SA, Australia

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[3]Basic Medical Sciences and Clinical Developmental Sciences, St. George's, University of London, London, United Kingdom

Basal laminas are specialized sheets of extracellular matrix that have critical roles in tissues and are important in diseases such as diabetes. Diabetes is associated with the deposition of additional layers of basal lamina in many organs. In bovine ovaries many follicles have additional layers of follicular basal lamina (1). To determine if such follicular basal laminas exist in women, ovaries from non-polycystic (PCO) (n = 42 follicles; n = 15 ovaries) and PCO women (n = 37 follicles; n = 3 ovaries) were examined by

electron microscopy. The follicular basal lamina of primary and secondary follicles was thicker than that of either primordial or antral follicles, as observed previously in bovine follicles (1). Most healthy antral follicles from both non-PCO and PCO ovaries had a single layer of basal lamina. Follicles with additional layers were also seen in both non-PCO and PCO. To further examine such follicles in an experimental system, oocytes were recovered from bovine small follicles (2-5 mm). A section of each follicle was fixed for histological assessment of follicle health and characterization of the follicular basal lamina. Oocytes underwent IVP, utilizing a novel single IVP system. Blastocyst development was examined on Day 8. A total of 211 oocytes were cultured, 69% were from healthy follicles and overall 32% developed to the blastocyst stage. Forty-three percent of oocytes recovered from atretic follicles (28/65) developed to the blastocyst stage, compared to only 27% (39/146) of oocytes from healthy follicles ($P < 0.05$). For healthy bovine follicles, the presence of a single layer of basal lamina was associated with significantly ($P < 0.01$) greater oocyte developmental competence (65% versus 28%), compared to healthy follicles with additional layers of basal lamina. These findings provide further evidence that the follicular environment is linked to oocyte competence.

(1) Irving-Rodgers HF and Rodgers RJ (2000) Ultrastructure of the follicular basal lamina of bovine ovarian follicles and its relationship to the membrana granulosa. J. Reprod. Fertil. 118, 221-228

009

THE STATIC AND IMMEDIATE EFFECTS OF NUTRITION ACT IN SYNERGY TO INCREASE FOLLICLE DEVELOPMENT IN MERINO EWES

C. Vinoles, B. Paganoni, J. T.B. Milton, D. Blache, M. A. Blackberry, G. B. Martin

School of Animal Biology, University of Western Australia, Crawley, WA, Australia

Forty Merino ewes were used in 2x2 factorial design: supplement (yes or no) and condition score (fat and lean). Ewes received 3 injections of prostaglandin (PG) 7 days apart and the supplement was fed from 2 days after the second PG until the third PG. Supplemented ewes received a diet that provided double their requirements for maintenance. Follicle development was monitored by ultrasound and blood was sampled daily to measure progesterone, FSH, oestradiol, insulin, leptin and IGF-I. The supplement promoted an increase in the number of 3-mm follicles ($P = 0.06$) and in the concentrations of insulin and leptin ($P < 0.001$). The concentrations of FSH were lower in supplemented than non-supplemented ewes ($P < 0.01$). Fat ewes developed more follicular waves and had higher concentrations of insulin, leptin and IGF-I ($P < 0.05$), while their FSH values tended ($P = 0.09$) to be higher than those in lean ewes. Leptin and insulin concentrations remained high in fat, supplemented ewes until the end of the supplementation period, whereas they decreased after Day 3 in lean ewes. Fat, supplemented ewes had 55% higher ovulation rate than lean, supplemented ewes ($P<0.05$). We concluded that higher concentrations of FSH and metabolic hormones in fat ewes will promote the development of more follicular waves, that superimposed on the dynamic changes in metabolic hormones promoted by the supplement, will stimulate the selection of more follicles into the wave, thus increasing ovulation rate.

010

withdrawn

011

EXPRESSION OF CONNEXINS 37, 40, AND 43 IN THE OVARIES OF THE BRUSHTAIL POSSUM, SHEEP, RAT, AND RABBIT

L. J. Haydon[1], S. M. Hrynuik[1], D. A. Heath[1], J. L. Juengel[1], D. C. Eckery[1,2], K. P. McNatty[1,2]

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[2]Cell Biology and Biotechnology, Victoria University, Wellington, Wellington, New Zealand

The maturation of an oocyte during ovarian follicular development involves a two-way communication between the oocyte and surrounding granulosa cells. Gap junctions are transmembrane channels consisting of proteins from the connexin (Cx) family that can facilitate communication between granulosa cells and the oocyte. During follicular growth, gap junctions have been identified between the oocyte and granulosa cells from the earliest stages of development. Many different members of the Cx family have been found in the ovary including Cx37, Cx40 and Cx43.

The aims of this study were to compare the ovarian cellular expression patterns of Cx37, 40 and 43 in 4 different species namely the brushtail possum, sheep, rat, and rabbit and to identify which of these connexins might be produced by oocytes. The reasons for selecting these species are that rat and rabbit ovulate many oocytes (5-12) and are spontaneous and reflex ovulators respectively, whereas sheep and possums ovulate 1-2 oocytes but have wide evolutionary separation. We used in situ hybridisation and immunohistochemistry (3-5 ovaries/species) to identify the cell-types in the ovary which expressed the connexins.

Cx37 mRNA and protein were present in oocytes of possums, sheep and rabbits throughout follicular development from the primordial stage and in oocytes of rats of early preantral follicles. In sheep and rabbit, Cx37 was localised in granulosa cells of some antral follicles. In all species, Cx37 was also localised to endothelial cells. Cx40 was localised to endothelial cells with little or no expression in other cell-types. In contrast, Cx43 expression was widespread in most ovarian cell-types. However, possums were the only species in which Cx43 was present in oocytes where it was present throughout all stages of follicular development.

We conclude that Cx37 is an important oocyte-derived gap junctional protein in a diverse range of species. Moreover, that although Cx43 is expressed in a wide range of ovarian cell-types in all species examined, it is not a commonly expressed Cx in oocytes.

012

ABNORMALITIES IN THE FEMALE REPRODUCTIVE TRACT OF FOLLISTATIN NULL MICE EXPRESSING A HUMAN FOLLISTATIN-315 TRANSGENE

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Follistatin primarily functions as an activin-binding protein. Alternative splicing of the follistatin gene results in the expression of two protein isoforms. The shorter form, follistatin-288, binds to heparin proteoglycans while follistatin-315 is a circulating form but their respective functions are unknown. Selective expression of the human FS315 isoform in transgenic mice (tghFS315) lacking the mouse follistatin gene results in fertile males and infertile females that showed copulatory plugs. This study characterizes the reproductive tract phenotype of tghFS315 females to determine why they are infertile.

Morphologically, the reproductive tracts of adult female tghFS315 mice lack a distinct infundibulum and have visibly shorter uterine horns and vagina. These mice also lack a urethral bud with the vagina and urethra sharing the same opening. Histological analysis reveals excessive leucocyte infiltration in the uterus and in vaginal smears of adult animals, indicative of severe inflammation.

Ovaries were collected from 8-10 week tghFS315 and wild type mice at 3.5 days post-coitum then serially sectioned at 3µm. The principal ovarian structures were counted in every 10^{th} section of each ovary and aggregated. This showed that tghFS315 mice have reduced follicle numbers and an absence of corpora lutea compared to wild type mice (Tb. 1).

Ovarian structure	Primordial follicle	Primary follicle	Secondary follicle	Antral follicle	Corpus luteum	Cyst
Wild type	115.8 ±16.1	102.8±22.5	118.3±10.6	117.3±19.1	88.5 ± 23.7	0.00 ±0.00
tghFS315	7.7 ± 6.2	1.0 ± 0.9	5.3 ± 2.5	0.00 ± 0.00	0.00 ± 0.00	25.7 ± 14.1

Table 1. Aggregate of ovarian structures observed in each 10^{th} section of wild type and tghFS315 ovaries at 3.5days post-coitum (mean ± SEM). Wild type n=4, tghFS315 n=3.

These data indicate that there are multiple causes of the female infertility in the tghFS315 mice including failure of corpus luteum formation, severe follicular depletion as well as inflammatory infiltration of an anatomically abnormal reproductive tract.

013

IN SILICO TRANSCRIPTIONAL PROFILING OF DIFFERENTIALLY EXPRESSED GENES IN RESPONSE TO BONE MORPHOGENETIC PROTEINS

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βBone morphogenetic proteins (BMPs) are members of the transforming growth factor beta (TGFβ) superfamily of proteins. They are multifunctional proteins that regulate growth and differentiation in many cell types and in many animal species ranging from insects to mammals. The bone morphogenetic protein family, including ligands, receptors, and binding proteins, has emerged as a central player in ovary physiology and female fertility. This study reports results from a microarray experiment investigating the specificity of BMP signalling in HEK 293T cells. Microarray results showed that when HEK293T cells were treated with BMP2, BMP4, BMP6, BMP7 for 4 hours, a common set of 12 genes were upregulated by all BMP ligands. Seven of these genes were identified as known BMP target genes, i.e. ID1, ID2, ID3 and ID4, Smad6 and Smad7 and MSX2. None of the genes were present in the TGFβ1 control set. A larger set of unique genes were identified as differentially expressed for each of the individual ligands. The major limitation of microarray technology is that only the genes present on the chip are able to be investigated. To overcome this, we employed *in silico* transcriptional profiling methodology for the 12 common genes upregulated by all 4 BMPs. A region spanning 1500bp upstream of the transcription start site was extracted for each of the identified genes and motif prediction and identification was carried out. Analysis of the predicted motifs identified four elements that may be involved in the regulation process of the studied genes. These motifs were employed to scan the entire human genome for their presence, with the aim of identifying novel genes not present on the array that are potentially regulated by BMP ligands. The top 5 significant genes were selected and tested for gene expression changes in response to each of the BMP ligands.

014

INTERLEUKIN 11 AND LEUKEMIA INHIBITORY FACTOR REGULATE THE ADHESION OF HUMAN ENOMETRIAL EPITHELIAL CELLS

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Implantation requires the attachment and adhesion of the blastocyst to a receptive endometrium. The factors that regulate the adhesion process are poorly understood. Interleukin (IL)-11 and leukaemia inhibitory factor (LIF) belong to the IL-6 family of cytokines and are obligatory for implantation in mice. IL-11 and LIF are are dysregulated in endometrial epithelium in women with infertility during the receptive phase. We hypothesized that IL-11 and LIF regulate endometrial epithelial adhesion to facilitate trophoblast attachment to the uterine epithelium. We examined the roles of IL-11 and LIF in epithelial cell adhesion. Primary human

endometrial epithelial cells (HEEC) and HES (an endometrial epithelial cell line) were treated without and with combinations of IL-11, LIF or phosphorylated (p)-STAT3 inhibitor (p-STAT3i), the main downstream target of IL-11 and LIF. We compared extracellular matrix and adhesion molecule mRNA expression between treatment groups by oligo-gene arrays (containing 96 genes) (n=3), and adhesion to fibronectin (n=3) and primary trophoblast cells (n=3). HEEC and HES expressed 25 genes in common, while 14 genes were restricted to HEEC and 3 genes to HES. Selected genes regulated ≥2 fold by LIF and IL-11 in both HEEC and HES were verified by real-time RT-PCR. Real-time PCR analysis confirmed that IL-11 stimulated integrin (IT) α 2, ICAM-1, CD44, TGF β 1 and TIMP-1 while IT β 1 was decreased ($p<0.05$) compared to controls. In contrast LIF significantly stimulated CD44 and TGF b 1 mRNA ($p<0.05$). IT α 2 and TIMP-1 protein was stimulated by IL-11 compared to controls ($p<0.05$). Adhesion of HEEC and HES to fibronectin or trophoblast cells was promoted by IL-11 and the effect was inhibited by p-STAT3i. While LIF promoted the adhesion of HEEC to fibronectin and trophoblast it was not significant. IT α v β 3, a marker of uterine receptivity, was not produced in high abundance by the cells. This is the first study to show that IL-11 and LIF regulate epithelial cell adhesive properties and suggests that both cytokines may render the endometrium receptive to the implanting blastocyst.

015

CREATING A CHIMERIC TGFB FAMILY RECEPTOR TO STUDY RECEPTOR FUNCTION

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Sheep with a point mutation in the bone morphogenetic receptor 1B gene (BMPR1B, also known as ALK6), exhibit an increased ovulation rate and increased fecundity. ALK6 is a type I transmembrane serine/threonine kinase receptor involved in the signalling cascade of TGFβ superfamily ligands, specifically: BMPs 2, 4, 6, 7 and 15; GDF5 and AMH. Upon ligand binding, complexes of type I and type II serine/threonine kinase receptors activate intracellular Smad signalling proteins which translocate to the nucleus to effect downstream gene transcription and expression. Signalling through ALK6 activates Smads 1, 5 and 8. In contrast, binding of ligand to the TGFβ receptor ALK5 activates Smads 2 and 3. We have constructed a 'chimeric' type I receptor consisting of the ectodomain of ALK6 fused to the endodomain of ALK5. In theory, this chimeric receptor should bind BMPs and signal through ALK5 in a specific manner allowing us to dissect out this signalling from background signals and provide a diagnostic tool specific for examining ALK6 binding. Mammalian cells (cos7, 293T) were transiently transfected with combinations of our chimeric plasmid DNA and either a BMP-responsive promoter reporter construct (BRE-luc) or a TGFβ-responsive promoter reporter construct (CAGA-luc). Various ligands, targeting the two pathways, were added to the cells and the effects on signalling assessed by measuring luciferase expression. Our results have supported our theory in that ligands binding to the ALK6 ectodomain were able to induce signalling through the ALK5 endodomain. Such chimeric receptors will provide a useful tool for examining specific parts of the TGFβ signalling pathway.

016

LOSS OF TGF-BETA SENSITIVITY LINKED TO LOW BETAGLYCAN EXPRESSION IN GRANULOSA CELL TUMOUR LINES.

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Ovarian cancers are the most common fatal gynaecological disease with granulosa cell tumours (GCTs) accounting for approximately 5% of these incidences. Mechanisms that govern a malignant transformation in granulosa cells are poorly understood but likely involve a disruption to the growth regulatory pathways, such as those mediated by inhibin and transforming growth factor-beta (TGFβ) .

This study aimed to investigate whether GCT cells were refractory to the growth-inhibitory effects of TGFβ and/or inhibin as a result of the loss of betaglycan, a TGFβ /inhibin co-receptor which governs cellular sensitivity to these ligands. The TGFβ -responsiveness of two human ovarian cancer cells lines of GCT origin, KGN and COV434, was examined.

Luciferase reporter and cell viability assays determined n either the COV434 nor KGN cell lines exhibited responsiveness to TGFβ1 or TGFβ2. Real-time PCR and ligand binding assays further demonstrated that although betaglycan mRNA was readily detectable in both lines low betaglycan ligand binding was observed. Furthermore, low levels of endogenous betaglycan were confirmed by immunostaining GCT cells using a betaglycan antibody. Transient transfection of both lines with a wildtype betaglycan expression plasmid restored responsiveness to TGFβ , indicating that aside from the loss of betaglycan, the TGFβ signalling pathway is intact in these lines. Finally, we established that an inhibitor of NFκB activation re-sensitises GCT cells to TGFβ resulting in reduced cell viability.

Collectively, these data establish the TGFβ-betaglycan signalling pathway as an important regulator of granulosa cell viability and further suggest that a deficiency in this pathway contributes to the pathogenesis of granulosa cell cancer.

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017

INSULIN-LIKE GROWTH FACTOR-II PROMOTES PLACENTAL FUNCTIONAL DEVELOPMENT AND FETAL GROWTH VIA THE TYPE 2 IGF RECEPTOR

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Treatment of the pregnant guinea pig with IGFs in early gestation enhances placental transport and growth of the fetus near term, but in part via different mechanisms (1,2). Exogenous IGF-II enhanced development of the placental labyrinthine (exchange region), while IGF-I, reduced maternal adiposity, near term. These differences are likely to stem from different interactions with IGF receptors, type 1 and 2 (IGF1R and IGF2R) and insulin receptor (InsR). While IGF-I acts predominantly through IGF1R, IGF-II can bind all three receptors. We assessed the role of the IGF2R in mediating actions of exogenous IGF-II in the mother by administering an IGF-II analogue, Leu^{27}-IGF-II, which only binds the IGF2R. IGF-II, Leu^{27}-IGF-II (1mg/kg/day sc) or vehicle were infused from days 20-38 of pregnancy and fetal growth, placental structure and transport of [^{3}H]-methyl-D-glucose (MG) and [^{14}C]-amino-isobutyric acid (AIB) and fetal plasma metabolites measured on day 62 (term=70 days) . Both IGF-II and Leu^{27}-IGF-II increased the proportion of placental labyrinth compared to vehicle (+9%). IGF-II and Leu^{27}-IGF-II also increased the volume of trophoblast and maternal blood spaces within the labyrinth (+28%, +40% respectively), and total surface area of trophoblast for exchange (+39%, +277%, respectively), compared to vehicle. Leu^{27}-IGF-II also reduced the barrier to diffusion (thickness of trophoblast) by ~50%, compared to vehicle and IGF-II. Both IGF-II and Leu^{27}-IGF-II increased fetal circulating amino acid concentrations (+137%, +339%, respectively) and placental transfer of MG to the fetus (+41%, +89%, respectively) compared to vehicle, with Leu^{27}-IGF-II also increasing AIB transport by 240% compared with vehicle and IGF-II. Both IGF-II and Leu^{27}-IGF-II increased the number of viable fetuses by ~25% and IGF-II increased fetal weight by 11%, compared to vehicle. In conclusion, maternal treatment with Leu^{27}-IGF-II or IGF-II in early gestation, induce similar placental and fetal outcomes near term. This suggests that maternal IGF-II availability in early gestation can act via the IGF2R to persistently enhance placental functional development and nutrient delivery and so promote fetal growth and survival near term.

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018

COMPLEX INTERACTIONS OF IGF-II, UPA UPA RECEPTOR AND PLASMINOGEN WITH OXYGEN IN TROPHOBLAST INVASION

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In early pregnancy, trophoblast cells invade and remodel uterine arteries to sequester a blood supply for the placenta. Impairments in this process have been implicated in common pregnancy complications. IGF-II promotes, and TGFβ1 inhibits, trophoblast invasion. We aimed to elucidate the molecular interactions between IGF-II, urokinase plasminogen activator (uPA), uPAR, IGF2R, TGFβ1 and oxygen that regulate trophoblast invasion. We cultured HTR8/SV-neo trophoblasts in RPMI1640 with 10% FCS with or without a Matrigel substrate in 20%, 5% or 1% oxygen for 96h. Cells were counted using Rose Bengal. Total RNA was extracted and subjected to RT-PCR to quantify IGF-II, uPA, uPAR, plasminogen, TGFβ1 and IGF2R mRNA relative to RpP0 rRNA. Matrigel invasion assays were used to determine the receptor that mediates IGF-II pro-invasive effects with either exogenous IGF-I, IGF-II or Leu^{27}IGF-II with uPA and plasminogen or 0.5% FCS in media alone in the 3 different oxygen atmospheres. Total and active TGFβ1 secretion were measured. Dose responses of specific inhibitors of uPA (amiloride) and plasmin (α2-antiplasmin) determined the relative roles of these proteases in invasion. Culturing HTR8/SV-neo trophoblast cells on Matrigel compared to plastic significantly increased their proliferation and prevented its exacerbation in response to low oxygen. Similarly, both the substrate and oxygen significantly affected trophoblast mRNA expression of all the genes quantified. Both IGF-II and Leu^{27}IGF-II, but not IGF-I, increased trophoblast invasion in a dose dependent fashion but this effect was most dramatic in 1% oxygen. Amiloride at higher doses, completely abolished the increased invasion observed in 1% compared to 5% and 20% oxygen, while α2-antiplasmin had similar but less potent effects, implicating these proteases in the mechanism by which low oxygen promotes trophoblast invasion. Both low oxygen and IGF treatment inhibited TGFβ1 activation but this was insufficient to increase trophoblast invasion. Our data suggest that exogenous IGF-II, via IGF2R, synergises with low oxygen to promote trophoblast invasion and inhibit activation of TGFβ1 by the plasminogen activator system in trophoblast cells.

019

INVOLVEMENT OF TTX-RESISTANT NA^+ CURRENTS AND PROTEIN KINASE C ON PRIMARY CULTURED SOMATOTROPES FROM GFP-GH TRANSGENIC MICE IN THE EFFECT OF GHRH

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Growth hormone (GH) secretion is primarily mediated by two hypothalamic hormones, GH-releasing hormone (GHRH) and somatostatin. It is well-known that GHRH depolarizes the cell membrane potential of somatotropes, leading to an increase in $[Ca^{2+}]i$ and GH secretion. Three major cation channels in somatotropes, Ca^{2+}, K^+, and Na^+, are involved in the regulation of cell excitability, which in turn regulates GH secretion. Na^+ channels mediate rapid increase in Na^+ permeability during the initial cell excitability. It has been suggested that GHRH increases the membrane Na^+ permeability via Na^+ channels which are not blocked by tetradotoxin (TTX-resistant or TTX-R), and that this may be mediated by cAMP pathways, leading to a depolarization of membrane potential and Ca^{2+} influx (1). This TTX-R Na^+ current has, however, not been characterized in somatotropes yet. In this study, we demonstrate the expression of TTX-R Na^+ current and its modification by GHRH in Green Fluorescent Protein (GFP)-GH transgenic mice somatotropes, using patch-clamp recording configuration. Application of GHRH (100 nM) directly onto the cell caused a significant increase in the TTX-R Na^+ current, which was reversible with removal of GHRH. The GHRH-induced increase in current was not affected by cAMP antagonist Rp-cAMP or PKA inhibitor KT5720 or H89. It was also not affected by cAMP analogues; 8-bromo cAMP, forskolin (adenylyl-cyclase activator) and IBMX (phosphodiesterase inhibitor) increased TTX-R Na^+ current but did not affect the action of GHRH. U-73122 (PLC inhibitor) totally abolished the TTX-R Na^+ current response to GHRH. PKC inhibitors, Gö-6978 and chelerythrine, also blocked the effect of GHRH. PKC activators, PDBu (phorbol dibutyrate) and PMA (phorbol myristate acetate), increased TTX-R Na^+ current, additional GHRH had no further effect on the current. These results suggest that the GHRH-induced increase in the TTX-R Na^+ current in mouse somatotropes is mediated by the PKC system. An increase in the TTX-R Na^+ current may depolarize membrane potential, enhance Ca^{2+} influx, and lead to GH secretion from somatotropes.

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020

BETA-ADRENERGIC REGULATION OF NORPHAN NUCLEAR RECEPTOR SIGNALLING: INSIGHTS INTO THE CONTROL OF METABOLISM

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Beta-adrenergic receptor deficient mice develop severe nutrient-induced obesity and display aberrant metabolic responses to temperature and diet. Recently, we established that beta-adrenergic receptor agonist treatment significantly induced the expression of the orphan nuclear receptor NOR-1 in type IIB dominant glycolytic muscle. We now demonstrate that beta2-adrenergic receptor agonist treatment significantly and transiently activated the expression of all three members of the orphan NR4A nuclear receptor subgroup [Nur77 (NR4A1), Nurr1 (NR4A2) and NOR-1 (NR4A3)] in type I oxidative red fiber dominant and type II glycolytic white fiber dominant muscle. Similarly, these changes are observed in an in vitro skeletal muscle cell culture model, and in other metabolic tissues including liver and white adipose. To elucidate the function of the 'NORphan' [NOR-1 (NR4A3)] in metabolism, we studied stable skeletal muscle cell lines transfected with NOR-1 siRNA. This analysis revealed distinct changes in lipid and carbohydrate utilization and oxidative vs anaerobic metabolism. Expression profiling of these cell lines and metabolic tissues (from mice treated with beta-adrenergic agonists) reveal NORphan signalling is involved in the regulation of distinct genes/pathways that modulate pyruvate utilisation, lipid and energy homeostasis.

021

MOLECULAR BASIS OF AROMATASE OVER-EXPRESSION IN OVARIAN GRANULOSA CELL TUMOURS

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Adult Granulosa cell tumours (GCT) are hormonally active, stromal cell neoplasms. The molecular basis of estrogen synthesis in GCT this remains unknown. Estrogen biosynthesis is catalyzed by aromatase. This study aims to investigate the mechanisms driving aromatase overexpression in GCTs. Real time quantification of transcripts in GCT (n=8), normal premenopausal ovaries (n=9) and GCT-derived KGN cell line revealed aromatase mRNA expression was ~ 17-fold higher in GCT than in normal ovaries and was driven by the up-regulation of the alternative proximal promoter, promoter II. Quantification of PII transcriptional activators orphan nuclear receptors Steroidogenic Factor-1 (SF-1) and Liver Receptor Homologue-1 (LRH-1) revealed LRH-1 mRNA was ~30-fold higher in GCT than in normal ovary, whilst SF-1 expression remained unchanged; suggesting that aromatase over-expression in GCT was driven by LRH-1. Transient transfection showed both recombinant SF-1 and LRH-1 can stimulate transcription and reporter induction was conferred by an AGGTCA motif at 130 base pairs. Although, affinity binding assays indicated both receptors had similar binding kinetics. EMSA and CHIP demonstrated the predominant binding activity in KGN nuclear extracts was SF-1, not LRH-1. To address this discrepancy, western blot analysis using KGN extracts were conducted. LRH-1 protein expression was undetectable, but was readily detected in MCF-7 cells, which express LRH-1 mRNA at ~80-100 fold lower levels of LRH-1 than

KGN. Immunohistochemistry co-localized aromatase and SF-1 to intra-tumoral stroma in all GCT patient tissue (n=5), whilst LRH-1 immunoreactivity was absent. Furthermore, sequence analysis of the LRH-1 transcript revealed no changes in the coding sequence. We are currently investigating the potential of microRNAs in the regulation of LRH-1 in GCTs.

022

WNT PATHWAY INHIBITORS ARE STRONGLY DOWN-REGULATED IN PITUITARY TUMOURS

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The aetiology of sporadic pituitary tumours is currently unknown. Wnt pathways have been implicated in the pathogenesis of a variety of human tumours but the role of these pathways in pituitary tumours needs elucidating.

The objective of the study was to identify genes involved in pituitary tumorigenesis.

Human pituitary tumours were collected at the time of surgery, snap-frozen and stored at -80°C. Microarray analysis using Affymetrix HG U133 plus 2.0 GeneChips identified down-regulation of several Wnt pathway inhibitors in pituitary tumours (n=20) versus normal pituitary controls (n=3). In particular, Wnt inhibitory factor 1 (WIF1) was down-regulated 91-fold, secreted frizzled-related protein 2 (sFRP) 20-fold, sFRP3 7-fold, and sFRP4 8-fold. Validation using quantitative PCR confirmed reduced WIF1, sFRP2 and sFRP4 mRNA expression in tumours (n=42) compared with normal pituitary (n=5) ($p < 0.001$, $p < 0.05$ and $p < 0.05$ respectively).

Down-regulation of WIF1 mRNA expression was the most striking result so was studied further. Assessment of WIF1 protein status was performed using immunohistochemistry with 76% of pituitary tumours (n=41) demonstrating absent or weak cytoplasmic WIF1 staining compared with strong staining in 92% of normal pituitary controls (n=13) ($p < 0.001$). To ascertain why WIF1 expression was reduced in pituitary tumours we assessed methylation status of the WIF1 promoter. Hypermethylation of tumours was demonstrated compared with normal pituitary controls ($p = 0.001$). The downstream central mediator of the canonical Wnt pathway is beta-catenin so immunohistochemistry for beta-catenin was performed. Abnormal nuclear beta-catenin accumulation was not observed in the pituitary tumours (n=70) but cytoplasmic staining weakly correlated with WIF1 and sFRP5 mRNA expression ($p = 0.058$ and $p = 0.053$).

In conclusion, three Wnt pathway inhibitors were identified as being down-regulated in all three pituitary tumour subtypes examined suggesting that the Wnt pathways are important in tumorigenesis. Our data supports that loss of WIF1 in pituitary tumours occurs as an early event, since it is common to all subtypes, and that further genetic events may explain the differences in tumour behaviour between the tumour subtypes.

023

MICRORNAS ARE DIFFERENTIALLY EXPRESSED BETWEEN MID AND LATE GESTATION IN THE MOUSE PLACENTA.

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MicroRNAs (miRNAs) are short, single-stranded, non-coding RNAs involved in the post-transcriptional repression of gene expression. MiRNAs bind to complementary sites in the 3'UTR of target mRNAs to repress or silence translation, which subsequently reduces transcript and/or protein levels. Placental functional development is characterized by dynamic and co-ordinated changes in expression of many regulatory and functional genes that drive invasion, differentiation and growth. These changes may arise in part from altered expression of miRNA regulatory networks. MiRNAs have been detected in the mammalian placenta but their patterns of expression throughout pregnancy have not been systematically characterized.

Thus in the current study, microarrays were used to compare miRNA gene expression in mid (day 13) and late gestation (day 18) murine placenta (term ~ 21 days).

Approximately 26% of all miRNAs examined were significantly upregulated and ~16% were significantly downregulated in the placenta in late compared to mid gestation ($p < 0.05$). Many upregulated miRNAs are members of polycistronic clusters, including several from an imprinted cluster found on human chromosome 14q32 and mouse chromosome 12 (mir-127, 136, 376a, 377). The miRNAs in this imprinted cluster are maternally-expressed and previous studies have shown that disruption of their expression impairs placental vascular development, disrupts labyrinth and junctional zone development and causes embryonic lethality [1] . Several miRNAs are also differentially expressed between the labyrinth (exchange region) and junctional zone in late gestation.

Many of the upregulated miRNAs and several of the downregulated miRNAs in the developing mouse placenta are predicted to target genes that regulate trophoblast differentiation and growth, such as CDX-2 and IGF-2, or genes that are involved in solute transport such as GLUT1. These findings suggest that miRNAs and the factors that influence their expression may have a role in the regulation of the functional development of the placenta and hence the fetal environment.

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024

ADIPONECTIN ACUTELY INCREASES INTRACELLULAR CALCIUM AND INSULIN SECRETION IN MIN6 CELLS

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An increase in adiposity predisposes to type 2 diabetes in which insulin resistance gives rise to hyperinsulinemia, in the early stages of the disease, that is later followed by failure of pancreatic β-cells to secrete insulin. Adipose tissue is now recognized as an active endocrine organ, secreting adipokines involved in energy homeostasis. Adiponectin is one of the recently discovered adipokines but, paradoxically, its levels are decreased in obesity. In skeletal muscle, adiponectin suppresses triglyceride accumulation and increases fatty acid oxidation, improving insulin signalling, and it suppresses glucose production in the liver, implicating adiponectin as an insulin sensitiser. The effects of adiponectin on pancreatic β-cells are not known. Our aim was to address this issue using Min6 cells, a murine pancreatic β-cell line. We established the presence of adiponectin receptors 1 (R1) and 2 (R2) mRNA in Min6 cells using real time PCR. R1 mRNA was 50 fold more abundant than R2 mRNA. Incubation with full-length adiponectin (2 μg/ml) for 24hr upregulated mRNA for R1 (2.46-fold) and R2 (1.67-fold), and increased insulin levels to 131 ± 4%. Acute (30min) application of full-length adiponectin (2 μg/ml) increased insulin levels, that was associated with an immediate (within 1min) increase in intracellular free calcium concentration $[Ca^{2+}]_i$. These adiponectin-induced increases in insulin and $[Ca^{2+}]_i$ as were comparable to those evoked by high glucose stimulation (Table, responses expressed as % of insulin and $[Ca^{2+}]_i$ in 3mM glucose). This increase in $[Ca^{2+}]_i$ was prevented by nifedipine, a blocker of L-type Ca^{2+} channels, and did not occur in Ca^{2+} free solution.

	Insulin	$[Ca^{2+}]_i$
Adiponectin (in 3mM glucose)	142 ± 14% (P=0.03)	185 ± 3% (P=0.001)
25mM glucose	161 ± 11% (P=0.01)	216 ± 8% (P=0.001)

In conclusion, adiponectin acts via its receptors in pancreatic β-cells to release insulin by increasing $[Ca^{2+}]_i$ via an increase in Ca^{2+} influx, revealing adiponectin as a potential novel therapeutic target in obesity-linked type 2 diabetes.

025

DIET-INDUCED OBESITY AND PRENATAL UNDERNUTRITION LEAD TO CENTRAL LEPTIN RESISTANCE BY DIFFERENT MECHANISMS

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Background : We have previously reported that prenatal undernutrition leads to a dysregulation of appetite suppressive effects through alterations in neuropeptide gene expression of whole hypothalami, which may be associated with central and peripheral leptin resistance [1] . In the current study, we expand our initial observations and investigate the neuroendocrine transcriptional response and leptin sensitivity in the arcuate nucleus (ARC) of rats exposed to prenatal undernutrition (UN) or a postnatal high-fat diet (DIO).

Methods : Pregnant Wistar rats were fed a standard chow diet either *ad libitum* (AD) or at 30% of AD intake throughout gestation (UN). At weaning, female offspring were fed either a chow [C], high fat [HF] (30% fat wt/wt) or calorie restricted [CR] (70% of standard chow intake) diet *ad libitum* for the remainder of the study. At 142 ± 5 days, AD and UN offspring received either recombinant rat leptin (2.5μg/g/day) or saline for 14 days, subcutaneously [2] .

Results : Using quantitative reverse transcription-polymerase chain reaction (qRT-PCR), we found that both NPY and ObRb mRNA expression in the ARC was significantly increased in UN animals ($P<0.05$), but remained unchanged in DIO animals when compared to control *ad libitum* chow-fed offspring. However, AgRP, was significantly increased in both groups (UN: $P<0.001$; DIO: $P<0.05$). Peripheral leptin treatment, in both UN and DIO animals, was ineffective in reducing NPY and AgRP mRNA expression, and had no effect on ObRb expression.

Conclusions : These findings suggest that prenatal undernutrition and diet-induced obesity lead to central leptin resistance through alterations in hypothalamic ARC neuropeptide mRNA expression and reduced sensitivity to leptin's anorexigenic effects, albeit by fundamentally different mechanisms.

(1) Ikenasio-Thorpe, B.A., et al., Prenatal influences on susceptibility to diet-induced obesity are mediated by altered neuroendocrine gene expression. J Endocrinol, 2007. 193(1): p. 31-7.

(2) Krechowec, S.O., et al., Prenatal influences on leptin sensitivity and susceptibility to diet-induced obesity. J Endocrinol, 2006. 189(2): p. 355-63.

026

A COMPARISON OF MOUSE AND HUMAN MRAPS: ACCESSORY PROTEINS FOR THE HUMAN MELANOCORTIN RECEPTOR 2 IN HEK293 CELLS

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Mutations in melanocortin 2 receptor accessory protein (MRAP) are implicated in approximately 20% of all cases of familial glucocorticoid deficiency (FGD), which results from defects in human melanocortin-2 receptor (hMC2R) functional expression. Two isoforms of *MRAP* exist in mice and humans, a long form (MRAP-α) and a short form (MRAP-β). Mouse MRAP-α trafficks the MC2R to the plasma membrane; where it is activated by its ligand, adrenocorticotrophin hormone (ACTH). We have tested mouse MRAP-β and the human MRAP-α and MRAP-β homologs for trafficking of the hMC2R to the plasma membrane in HEK293 cells. Human and mouse MRAP-α share a highly conserved N-terminus and transmembrane domain but a divergent C-terminus. While mouse and human MRAP-β also share conserved N-termini and divergent C-termini, mMRAP-β lacks a transmembrane domain. Unlike the other melanocortin receptor subtypes, the hMC2R does not functionally express in heterologous cell types due to a lack of receptor trafficking to the plasma membrane. We have shown that mouse MRAP-α, when either transiently or stably expressed in HEK293 cells enables the hMC2R to couple to adenylyl cyclase in response to stimulation with A CTH . In the absence of mouse MRAP-α, no functional expression is observed. In contrast to mMRAP-a, mMRAP-β does not promote coupling of hMC2R to adenylyl cyclase in HEK293 cells. We propose MRAPs require a transmembrane domain to interact with hMC2R. We have cloned α and β isoforms of hMRAP from testes and ovary mRNA, respectively. Human MRAP-α promotes hMC2R coupling to adenylyl cyclase following stimulation with ACTH in HEK293 cells with an identical EC_{50} (1×10^{-10}M) to that seen with mMRAP-α. This is the first comparison of the relative effect of mouse and human MRAP-α on hMC2R expression. We are currently testing the effect of human MRAP-β on hMC2R expression. Future work will focus on understanding the mechanism by which MRAPs traffick the hMC2R to the plasma membrane.

027

REGULATION OF ENDOTHELIN-1 IN HUMAN ENDOTHELIAL CELLS BY SEX STEROIDS AND ANGIOTENSIN II.

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It is well documented that there are gender differences in the incidence and patterns of cardiovascular disease. Males have a higher incidence of cardiovascular disease than premenopausal women. We have shown previously that testosterone but not oestradiol can increase the number of human aortic endothelial cells (HAEC) secreting the vasodilator and cardio-protective peptide, adrenomedullin [1]. Here, we have investigated whether oestradiol and testosterone can directly influence the secretion of endothelin-1 (ET-1), a powerful vasoconstrictor and growth-promoting peptide, from HAEC. Several papers have reported that oestradiol can decrease ET-1 secretion from endothelial cells whereas the actions of testosterone are less clear.

HAEC were incubated in serum free DMEM with or without testosterone (3.5-3500 nM), oestradiol (0.0037-3700 nM) and/or angiotensin II (0.0001-1 μM). The number of cells secreting ET-1 were counted after immunocytology on a protein binding membrane, and RT-PCR was performed to measure ET-1 mRNA.

We found that oestradiol did not increase the number of cells secreting ET-1 under basal conditions but decreased the number of secreting cells stimulated with angiotensin II ($n=5$, $P<0.01$). In contrast testosterone dose dependently stimulated the number of cells secreting ET-1 to 136% with 350 nM ($n=4$, $P<0.05$) and to 162% of control with 3500 nM testosterone ($n=4$, $P<0.05$). Furthermore, testosterone up regulated ET-1 mRNA at 1-3 hours to 120% ($n=9$, $P<0.05$) of control but reduced ET mRNA at 12 hours to 72% ($n=7$, $P<0.05$) of control. These results indicate that ET-1 mRNA, ET-1 peptide production, and adrenomedullin peptide secretion, are altered in a similar fashion in HAEC under our conditions of study. We conclude that there is potential for a coordinated modification of vasoactive peptide production by the sex steroids in endothelial cells.

(1) Pearson LJ et al. (2006) Regulation of adrenomedullin release from human endothelial cells by sex steroids and angiotensin-II. J Endocrinol 191:171-177.

028

DOES RFRP-3 SUPPRESS PULSATILE LH SECRETION IN THE RAT?

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The recently discovered gonadotropin-inhibitory hormone (GnIH) is released by hypothalamic neuroendocrine neurons and, along with gonadotropin-releasing hormone (GnRH), regulates fertility in birds. GnIH soma are localized close to the dorsal medial hypothalamus and project their fibres to the median eminence (ME) from where the neuropeptide is released to inhibit pituitary luteinizing hormone (LH) secretion. In contrast, studies on mammals have shown a virtual absence of fibres in the external zone of the ME that express the mammalian GnIH homologue, RFamide-related peptide-3 (RFRP-3). We therefore hypothesised that RFRP-3 acts via the hypothalamus to inhibit the tonic pulsatile and preovulatory surge modes of GnRH and LH secretion in rats. This is supported by previous work in mammals that shows close contact of RFRP fibres and GnRH cell bodies. We used LH pulse frequency as an index of GnRH pulse frequency. Female ovariectomized rats with slow release, low dose estradiol implants were chronically fitted with atrial and icv cannulas. Atrial blood samples were collected every ten minutes for four hours during which a

bolus icv injection of either RFRP-3 (500ng or 25 µg) or vehicle (n=4/group) was administered. Plasma concentration LH and pulse frequency was measured using radioimmunoassay. Pre-injection LH pulse frequency and amplitude averaged 0.85 ± 0.24 pulses/hr and 0.62 ± 0.20 ng/ml respectively; this was not significantly affected by either dose of RFRP-3. In contrast, in previous work icv RFRP-3 infusion significantly reduced the percentage of GnRH neurons activated (measured by Fos coexpression) during an estradiol-induced LH surge. Thus, while RFRP-3 may act centrally to modulate this preovulatory event, the current data do not support an action of RFRP-3 in regulation of GnRH pulse frequency.

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029

ROLE FOR FOLATE TO INCREASE MILK PROTEIN PRODUCTION

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Increasing milk protein production is a major goal of the dairy industry. Using functional genomics to exploit animal models with extreme adaptation to lactation is an alternative approach to identifying key genes that specifically regulate protein synthesis in the mammary gland. For example, milk protein is the only component of milk synthesized de novo in the mammary gland of the Australian Fur Seal. The tammar wallaby increases milk protein production in the latter half of lactation. Microarray analysis of mammary tissues from the pregnant and lactating seal, and mammary tissue from the tammar at early and late lactation were compared with the same analysis in the pregnant and lactating dairy cow. The number of genes identified across arrays is only approximate due to redundancy on the arrays (number of probes and number of genes are different), non-symmetric mapping between species, dependence upon threshold for mapping and sequence quality and reliance on unigene gene assemblies. We have concluded that 26% of tammar ESTs from the normalised library (not genes) can be mapped across the three species. Nine genes were differentially regulated (at least 2-fold) in the seal, wallaby and cow mammary glands. This candidate gene list indicates folate metabolism may be a crucial regulatory point of milk protein synthesis in mammary epithelial cells. Research in human, rodent and monkey cell lines show folate uptake and metabolism is regulated at multiple levels within the cell. We are currently evaluating three bovine in vitro models; mammospheres on a commercially produced murine matrix (Matrigel), mammospheres on their own secreted matrix and a bovine mammary epithelial cell line, BME UV1. This work will allow us to better understand the complexities of folate metabolism at the cellular level of the mammary gland, and its potential role to increase milk protein production at the levels of milk protein gene transcription and milk protein synthesis.

030

THE RELATIONSHIP BETWEEN PLASMA PROGESTERONE, CONCEPTUS DEVELOPMENT AND GESTATION LENGTH IN THE STRIPE-FACED DUNNART, *SMINTHOPSIS MACROURA*.

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The plasma progesterone profile of a dasyurid, *Antechinus agilis*, has been shown to be related to conceptus development, with periods of rapid development corresponding to elevated plasma progesterone. Distinctive daily body weight changes found during the gestation period in smaller dasyurids (e.g. *Dasyuroides byrnei)* also correlate with the plasma progesterone profile, and are thought to reflect uterine weight changes due to mitosis or oedema. This study aimed to (1) investigate the relationship between the rate of conceptus development and plasma progesterone concentration in another dasyurid, *Sminthopsis macroura*; (2) confirm that body weight changes reflect uterine weight; and (3) identify changes in progesterone concentration, uterine weight, body weight or gestation length associated with conceptus number.

Daily monitoring of body weight and vaginal cell concentration in urine samples reliably identified oestrous cycling, allowing collection of staged blood and tissue samples, conceptuses, and body and uterine weights. A daily mitotic index was calculated for the luminal and glandular uterine epithelium during the oestrous cycle.

For the first time in a polyovular marsupial, we found that increased conceptus number was associated with significantly higher progesterone concentration during specific developmental stages. The plasma progesterone profile was also associated with the rate of development in this species, and can be used to predict developmental timing in marsupials. Mitotic remodelling of the uterine epithelium occurred during the pre-ovulatory period. This study also identified epithelial mitosis associated with implantation for the first time in a marsupial. Uterine weight was correlated with daily body weight and was associated with the epithelial mitosis initiated during the peri-ovular period. Normal gestation length in *S. macroura* is 10.7 days, but gestations of between 9 and 13 days were identified. Correlations between conceptus development, plasma progesterone, uterine and body weight suggest that a reduction in gestation length is due to a reduced period of arrest at the 4-cell stage, and a more rapid period of definitive blastocyst expansion.

031

MILK YIELD IN EWES DURING MID- TO LATE LACTATION IS NOT AFFECTED BY PREGNANCY

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Dairy sheep are not usually milked when they are pregnant. When a year round supply of milk is required, ewes may be mated while they are lactating but it is not known if pregnancy reduces either milk yield or the persistency of lactation. To test the hypothesis that pregnancy alters milk yield, East Friesian crossbred ewes were ranked according to milk yield and ewes producing > 1000mL/day at day 90 of lactation were allocated into two groups, one of which (n = 91) were mated while the other group (n = 102) was not. Pregnancy was diagnosed 40 days later and non-pregnant ewes belonging to the mated group were removed from analysis. Milk volumes were recorded at fortnightly intervals until daily production fell below 500mL/day or until the experiment was discontinued after 184 days of lactation. Time (measured in days) affected mean daily milk yield (P = 0.001) and accounted for large amounts of the variation in daily milk yield. There was an interaction between time and treatment (P = 0.001) and significant differences (P < 0.05) between treatments in mean daily milk yield were observed at days 157, 173 and 184 of lactation. Of the 91 pregnant ewes and 102 non-pregnant ewes studied, there were differences in the number of ewes dried off within treatments at day 173 (41 pregnant ewes verses 23 non-pregnant ewes, P = 0.008), and day 184 of lactation (64 pregnant ewes verses 42 non-pregnant ewes, P = 0.006). Pregnant ewes were milked for fewer days than non-pregnant controls (172 ± 2.9 days and 181 ± 2.6 days respectively, P = 0.022) but produced similar quantities of milk (246 ± 7 L and 259 ± 7 L respectively, P = 0.193). We conclude the effects of pregnancy are not pronounced and are not evident until day 82 of pregnancy. *This work was funded by the RIRDC.*

032

A NOVEL MOUSE MODEL FOR ENDOMETRIAL MACROPHAGE EVALUATION IN EARLY PREGNANCY

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A major contributing factor in the success or failure of pregnancy is the quality of the relationship established between the embryo and the mother during the first days after conception, which is largely determined by the receptivity of the endometrial environment in which the embryo implants. Inflammatory cells recruited into the uterus in response to seminal plasma stimulation in early pregnancy have a role in 'conditioning' the endometrium to allow optimal implantation, fetal growth and placental development. Macrophages comprise the major population of cells recruited. The CD11b-DTR transgenic mouse has CD11b promoter-driven expression of the monkey diphtheria toxin (DT) receptor. The i.p. administration of nanogram doses of DT results in systemic ablation of macrophages. It has been shown that the number of peritoneal and tissue macrophages are substantially reduced within 24-48 hours following DT administration. We have shown that complete ablation of uterine macrophages can be achieved within 48 h of administration of DT. Using this model, the effect of endometrial macrophage removal during the pre-implantation phase of pregnancy was evaluated. Ablation of macrophages during early pregnancy (day 0.5 or day 3.5 post coitus) caused complete pregnancy loss in CD11b-DTR mice compared with wild-type (WT) control mice. Zero out of seven CD11b-DTR mice were pregnant following the administration of DT on day 0.5, while five out of nice WT mice retained their pregnancies, as assessed on day 7.5 of pregnancy. Similarly in mice treated with DT on day 3.5 of pregnancy, zero out of four CD11b-DTR mice were pregnant, compared with four out of eight WT mice. We conclude that DT-mediated ablation of endometrial macrophages is inconsistent with generation of uterine receptivity and successful embryo implantation. This model therefore provides a valuable tool for evaluating the immune and tissue remodelling functions of endometrial macrophages early in pregnancy in facilitating embryo implantation.

033

PLACENTAL P-GLYCOPROTEIN (*ABCB1*) EXPRESSION IS REDUCED BY GLUCOCORTICOIDS DURING LATE GESTATION IN THE RAT

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Fetal glucocorticoid excess programs adverse outcomes in adult offspring, including hypertension, obesity and insulin resistance. Access of maternal glucocorticoids to the placenta and fetus is regulated by the placental 11β-HSD enzymes, but recent studies show that glucocorticoid activation of placental GR is also restricted by the product of the multidrug resistance gene, P-glycoprotein (*Abcb1*)[1]. However, the expression and regulation of placental *Abcb1 in vivo* remain poorly understood. The current study measured rat placental expression of the two *Abcb1* genes (*Abcb1a* and *Abcb1b*) in normal pregnancy and following altered exposure to progesterone or glucocorticoid. Placentas were collected and dissected into their morphologically- and functionally-distinct zones on days 16 and 22 of normal pregnancy and after either dexamethasone treatment (0.75 μg/ml in drinking water from day 13) or ovariectomy (day 16) plus full estrogen and partial (to approximately one third) progesterone replacement. Basal and labyrinth zone expression of *Abcb1a* and *Abcb1b* mRNA were determined by qRT-PCR. Labyrinthine expression of both *Abcb1a* and *Abcb1b* markedly exceeded that in the basal zone (6-8 fold, $P<0.001$), and expression was similar between days 16 and 22. Dexamethasone reduced *Abcb1a* expression by 44% ($P<0.04$) only in the labyrinth zone at day 22, but had no effect on *Abcb1b* levels. Partial progesterone withdrawal had no effect on expression of *Abcb1a* or *Abcb1b* mRNA in either zone on day 22. This study demonstrates that placental expression of *Abcb1a* and *Abcb1b* was relatively constant over the final third of rat pregnancy and was unaffected by a premature reduction in progesterone. Increased glucocorticoid exposure, however, decreased labyrinthine *Abcb1a* expression, potentially decreasing the effectiveness of the placental glucocorticoid barrier.

(1) Mark PJ, Waddell BJ (2006) Endocrinology 147:5147

034

TRANSFORMING GROWTH FACTOR BETA IS A MAJOR CERVICAL SIGNALING FACTOR IN HUMAN SEMINAL PLASMA

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After intercourse, seminal plasma interacts with cervical cells to induce a local inflammatory-like response, influencing the female tract immune response to sperm and sexually transmitted pathogens. In women, the response is characterised by up-regulated expression of several pro-inflammatory cytokines including GM-CSF, IL-1a and IL-6, and chemokines IL-8, MIP-3a and MCP-1, followed by recruitment of macrophages, dendritic cells, granulocytes and lymphocytes into the cervical tissue. The identity of the key signalling agents in human seminal plasma is unclear. In mice, TGFb family members are key active factors in seminal plasma, and human seminal fluid contains abundant TGFb1, TGFb2 and TGFb3. The purpose of this study was to investigate whether any or all of these TGFb isoforms are active mediators of seminal plasma signalling in the cervical inflammatory response. Primary ectocervical epithelial cells or immortalised Ect1 cells were incubated with recombinant human TGFb1, TGFb2, or TGFb3.. The effect of seminal fluid and TGFb on epithelial cell inflammatory cytokine gene expression was analysed by Affymetrix microarray and qRT-PCR, and epithelial cell supernatants were analysed by commercial ELISA to quantify GM-CSF, IL-1a, IL-6, IL-8, MIP-3a and MCP-1 production. Microarray analysis showed that TGFb1 and seminal plasma both induced expression of inflammatory response, cytokine-signaling and TGFb pathway gene families. Each of the three TGFb isoforms mimicked seminal plasma and were comparable in their capacity to stimulate >5-fold increases in both GM-CSF and IL-6 secretion and mRNA expression in a dose-responsive manner, while addition of TGFb neutralising antibodies inhibited seminal plasma-induced increases in these cytokines. However unlike seminal plasma, TGFb was unable to stimulate IL-1a, IL-8, MIP-3a or MCP-1 production. These results demonstrate that each of the three TGFb isoforms operate in synergy to provide the major active constituents of human seminal plasma, mediating many of the pro-inflammatory cytokine responses elicited by seminal plasma in cervical cells. However signaling factors in addition to TGFb must exist and these remain to be identified.

035

THE ANGIOGENESIS INHIBITOR CALRETICULIN IS INCREASED IN MATERNAL BLOOD WITH PREGNANCY AND PRE-ECLAMPSIA

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The human pregnancy disorder of pre-eclampsia is hypothesized to involve widespread activation of maternal endothelial cells by factors that are released from the placenta into the maternal circulation. Calreticulin is a calcium-binding molecular chaperone that promotes folding and quality control in the endoplasmic reticulum via the calreticulin/calnexin cycle, as well as other functions including regulation of calcium homeostasis. Extra-cellular calreticulin inhibits endothelial cell proliferation *in vitro* and inhibits angiogenesis in mice *in vivo* (Pike et al, Blood, 94, 1999, 2461-8). Little is known about its role in human pregnancy and pre-eclampsia. The aims of this work were to identify calreticulin release from human placenta *in vitro* and to measure maternal plasma calreticulin throughout pregnancy and in pre-eclampsia. Western blot using polyclonal rabbit anti-human calreticulin measured calreticulin in maternal plasma and in the maternal effluent from human *in vitro* perfused placental cotyledons. The mean (±SEM) concentration of calreticulin was 0.84±0.12µg/ml for the effluent from 6 placentas and 1.80±0.33µg/ml for maternal plasma from the same pregnancies. There was a significant increase in calreticulin in plasma in term pregnant women (mean arbitrary density/0.5µl, 193.1±55.0, n=12, un-paired t-test $p<0.05$) compared with non pregnant women (40.2±16.0, n=12), however there was no difference in calreticulin in plasma from 8 women who had uncomplicated pregnancies and were sampled at first trimester (10.1±2.3weeks), second trimester (24.0±0.0weeks) and at term (37.5±1.1weeks). The mean arbitrary density/0.5µl was 778±230, 488±100 and 602±113 respectively (ANOVA $p>0.05$). In addition, there was a significant increase in calreticulin in plasma from pre-eclamptic women (1613±125, n=10, un-paired t-test $p<0.05$) compared to gestation-matched, normotensive controls (1053±172, n=12). These results indicate that the placenta may be a major source of calreticulin in the maternal circulation, that calreticulin increases in maternal blood early in pregnancy (possibly before 10 weeks), that it remains elevated throughout normotensive pregnancy and that it is further increased with pre-eclampsia. Calreticulin may play a role in changes in maternal cardiovascular function in pregnancy and pre-eclampsia.

036

MACROPHAGE REGULATION OF EMBRYO ADHESION MOLECULE EXPRESSION IN HUMAN ENDOMETRIAL EPITHELIAL CELLS

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The uterine endometrium undergoes dramatic structural and functional changes to allow the embryo to attach and implant in early pregnancy. The molecular processes occurring at implantation are critical in controlling early trophoblast invasion and ensuring adequate placental development. We hypothesise that macrophage populations resident in the endometrial tissue influence local cell communication and tissue remodeling events to promote uterine receptivity for embryo implantation. In our previous mice study we have shown that STAT-3 is a key regulator in the implantation window. In this study we have investigated the role of macrophages and their secreted products in the regulation of epithelial cell expression of adhesion and anti-adhesion molecules and the significance of STAT-3 signal transduction in macrophage-epithelial signaling.

Endometrial epithelial RL95-2, HEC1A and Ishikawa cell lines were co-cultured alone or in the presence of LPS- or PMA-activated or resting human monocytes separated spatially using transwell inserts. Expression of several genes encoding adhesion and anti-adhesion molecules, glycosylation enzymes, and steroid hormone receptors were measured by quantitative real-time RT-PCR using SYBR green chemistry. Ulex europaeus (UEA1), which is an L-fucose-binding lectin and has affinity with H type2 (0 type antigen) in fucosyltranslation, was analyzed by flow cytometry. The amount of activated STAT-3 was measured in nuclear protein extraction from endometrial epithelial cells.

Integrin beta 1, beta 3 and estrogen receptor beta mRNA expression were increased by PMA-stimulated monocytes. Fucosyl transferase (FUT) 1 and FUT2 mRNA levels were increased by co-culture with LPS-stimulated monocytes and resting monocytes. PMA-stimulated monocytes, LPS-stimulated monocytes and/or resting monocytes increased the population of UEA1 positive cells in epithelial cells. STAT-3 activity was significantly changed by co-culture with LPS- or PMA-activated or resting human monocytes.

These results suggest that macrophage-derived STAT-3 might facilitate development of an implantation-receptive endometrium via regulation of embryo adhesion molecule synthesis in epithelial cells. Dysregulation in the phenotypes or abundance of uterine macrophages might provide an explanation for some forms of primary unexplained infertility.

037

IMPLANTATION RATES IN MOUSE FOLLOWING TREATMENT WITH LIPIODOL

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Reports of a possible fertility-enhancing effect of a hysterosalpingogram (HSG) using oil-soluble contrast media (OSCM) first emerged more than half a century ago, followed by various trials showing conflicting results. However, a recent randomised-controlled trial has confirmed the effectiveness of Lipiodol (an OSCM comprising iodised poppy seed oil) infusion during HSG for couples with unexplained infertility . The mechanism by which Lipiodol HSG enhances fertility is not yet known. Lipiodol is composed of approximately 40% iodine in poppy seed oil. In turn the poppy seed oil contains primarily ethyl linoleate (a polyunsaturated fat) and in Lipiodol four molecules of iodine are chemically bonded to the ethyl linoleate component of the poppy seed oil. The aim of this study was to investigate the effects on blastocyst implantation rates, of the components of Lipiodol (i.e. the iodine or the oil) compared to the entire formulation (intact Lipiodol).

Female CD1 mice, aged 6–8 weeks, in pro-oestrous were anaesthetised and a lower abdominal laparotomy performed to expose a uterine horn. 50 µl of treatment fluid was injected into the exposed horn. The mice were treated with; Lipiodol, ethyl linoleate, Xenetix (a water-based iodised contrast medium) or saline. After being allowed to recover for 14 days, the female mice were mated. On day 8 post coitus, the pregnant females were euthanised and the uteri examined for the number of implantations.

Lipiodol treated mice had a slightly higher number of implantation (7.1) compared to the other control groups (5.3 – 6.9). However, the differences were not statistically significant.

This study investigated the effects of intra-uterine Lipiodol on the litter size of treated mice but Lipiodol treatment did not significantly increase implantation rates in this model.

038

THE DECREASE IN MYOMETRIAL RELAXIN RECEPTOR (LGR7) EXPRESSION AT THE END OF GESTATION IN THE RAT IS DRIVEN BY THE FETAL-PLACENTAL UNIT AND NOT MATERNAL PROGESTERONE

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Relaxin is thought to inhibit myometrial contractions in pregnancy. This action is mediated by a Type C GPCR, Lgr7. In mice, Lgr7 is localised to the myometrium, with a significant decrease in receptor expression at term. The factors responsible for Lgr7 down-regulation are unknown, but could include progesterone withdrawal or increasing uterine stretch. To address this question, we investigated the effects of: i) RU486 treatment, and ii) the absence of fetal-placental unit(s) on myometrial Lgr7 expression at different stages of gestation.

The gestational profile of myometrial Lgr7 expression was first established in pregnant and postpartum (PP) rats. The second study treated rats with RU486 (10 mg/kg), or vehicle on day 19 of gestation and myometrial tissue was collected 24 h later. A third group of rats had one ovarian tube ligated prior to mating and tissues were collected from gravid and non-gravid horns at various gestational stages and day 1 PP. The tammar wallaby has separate gravid and non-gravid uteri, and is a natural unilaterally pregnant animal. Myometrium was collected from paired uteri at different stages of gestation. Gene expression was analysed by quantitative PCR (1).

Myometrial Lgr7 expression was significantly reduced in late gestation in the rat but not tammar wallaby. In the rat there was a dramatic increase in Lgr7 postpartum so that receptor expression returned to non-pregnant levels. Administration of RU486 on day 19 of gestation caused a significant decrease in myometrial Lgr7, suggesting progesterone withdrawal contributes to the preterm decrease in Lgr7. Lgr7 expression was lower in the gravid uterus compared to the non-gravid uterus in both rats and tammar wallabies, demonstrating a negative effect of the fetal-placental unit on myometrial Lgr7 expression.

Data in the rat show that the fetal-placental unit and maternal progesterone have opposing actions in regulating myometrial Lgr7. The decrease in myometrial responsiveness to circulating relaxin may be needed to activate, rather than suppress, contractile-associated proteins in late gestation.

(1) Vodstrcil et al. 2007 Reprod Fert Dev 19: 530-8

039

AGE AND SEX SPECIFIC REGULATION OF THE GROWTH AXIS DURING DEVELOPMENT IN THE TAMMAR WALLABY: A MODEL TO STUDY MAMMALIAN FETAL GROWTH AND DEVELOPMENT

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Growth hormone is essential for post-natal growth and its actions are triggered by the upregulation of hepatic growth hormone receptors (GHRs) after birth in eutherian mammals. In utero, fetal growth is largely driven by the availability of nutrients that control the relative levels of insulin and insulin-like growth factors (IGFs). This study examined expression of hepatic GHR, IGF-1, IGF-2 and IGF binding protein-3 (IGFBP-3) from late fetal stages of pregnancy through pouch life to weaning using the tammar wallaby. The advantage of the tammar wallaby is that it gives birth to an altricial young that is only 0.1% of adult female size, essentially an exteriorized fetus. After 9 months of pouch life the young exits the pouch, a time equivalent to birth in precoial eutherian mammals.

We cloned GHR, IGF-1, IGF-2 and IGFBP-3 using RT-PCR and used quantitative PCR to determine the relative change in expression of these genes in male and female fetuses one day before birth and young at 15, 45, 70, 100, 150 and 250 days (d) post-partum (pp) and adults (n=7-10 per stage). There was significant protein homology between tammar genes and those of mouse and man (62 and 68% for GHR, 79 and 85% for IGF-1, 63 and 65% for IGF-2, 69 and 71% for IGFBP-3 respectively). Hepatic GHR and IGF-1 expression increased gradually in both males and females from birth to d150pp, after which levels reached a plateau (ANOVA $P<0.0001$). IGFBP-3 expression rose to a peak about d70-100pp then fell at later ages of lactation and in adults, with generally lower levels in females than males (ANOVA $P<0.01$ for age and $P<0.05$ for sex). Males and females had similar levels of IGF-2 expression with no significant trend with age or sex (ANOVA $P>0.05$). The age-related changes in expression of these genes probably reflects a conserved role in development, while the sex-specific differences may correlate with some of the growth and developmental differences observed between males and females.

040

MARSUPIAL WT1 HAS A NOVEL ISOFORM AND IS EXPRESSED IN BOTH SOMATIC AND GERM CELLS IN THE DEVELOPING OVARY AND TESTIS

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The Wilms tumour 1 gene is essential for the formation of the mouse and human urogenital systems. We characterised this gene and examined its expression throughout gonadal development in a marsupial the tammar wallaby. WT1 protein was detected in the Sertoli and granulosa cells of the developing testis and ovary respectively. There was also strong immunostaining in the germ cells of both males and females at all stages of gonadal development. In the adult gonads WT1 appears to be dynamically regulated during

spermatogenesis and oogenesis. Tammar WT1 has a novel isoform in which a portion of exon 1 is removed, partially deleting the RNA recognition motif (RRM). Despite its removal, WT1 still localised to RNA rich regions of the oocyte including speckled bodies within the nucleus, in the nucleolus and the perinucleolar compartment. This suggests that the RRM is not required for WT1 co-localisation with RNA. This is also the first report of WT1 in association with the perinucleolar compartment, important for RNA metabolism. Our data suggest that WT1 has a conserved function in both the somatic and germ cell lineages of the gonads of marsupials.

041

DOES PROLACTIN PLAY A ROLE IN REPRODUCTION IN THE BRUSHTAIL POSSUM?

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Prolactin (Prl) is known to play important roles in reproduction, in particular ovarian function and lactation in many species of mammals. In the brushtail possum *(Trichosurus vulpecula)* Prl has been shown to be important for the latter stages of lactation[1], however what role it might play in ovarian function is unknown. In female possums, a distinct bi-phasic preovulatory Prl surge occurred 3h prior to, and coincident with the preovulatory LH surge. Following ovulation, Prl secretion was suppressed for up to a week[1]. Moreover, widespread expression of Prl receptor (PrlR) mRNA was observed in the ovary, particularly in granulosa and interstitial cells, and corpora lutea[2]. The aims of this study were to determine the levels of mRNA expression of Prl in the pituitary gland at different stages of the oestrous cycle and determine the effects of Prl on granulosa cell function in the possum. Pituitary glands were collected from adult female possums (n=5/group) during the early and late follicular, and early and mid luteal stages of the oestrous cycle. Expression levels of Prl mRNA were determined by quantitative PCR using Taqman probes. For the cell culture studies, granulosa cells were collected from follicles between 1-2.5mm diameter and treated with 10 μg/ml Prl or media alone. Following treatment, progesterone levels in the culture media were determined by radioimmunoassay. Mean mRNA expression levels of Prl were lower during the luteal phase ($P<0.05$), which coincided with the suppressed levels of Prl in plasma as was previously reported during this time[1]. Treatment of granulosa cells with Prl resulted in a marked increase ($P<0.001$) in progesterone in the culture media. The regulation of Prl mRNA expression in the pituitary gland during the oestrous cycle and the stimulatory effects of Prl on progesterone production by granulosa cells provide further evidence that Prl may play a fundamental role in ovarian function and in particular steroidogenesis in the brushtail possum.

(1) Crawford et al., 2006 J Endo 190, 295-305.
(2) Juengel et al., 2000 NZ Endo Soc Abstract

042

MATERNAL INFLUENCE ON SEX IN A LIVE-BEARING GEKKOTAN LIZARD FROM SOUTHERN NEW ZEALAND

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Mechanisms of sex determination among reptiles show enormous variety: genotypic and environmental mechanisms and combinations thereof are reported. Temperature-dependent sex determination is well known in egg-laying species and reported in live-bearing lizards. Research on different lineages is important to establish evolutionary patterns of maternal influence on sex determination and to assist with conservation issues, including captive management and impacts of climate change in threatened species. We investigated clutch (litter) sex ratios in the common gecko Hoplodactylus maculatus from Otago, a New Zealand endemic within the Diplodactylidae, a family that likely has Gondwanan origins. This long-lived (40+ years), live-bearing gecko has a maximum clutch size of two; thus, if two neonates are produced, embryonic mortality is eliminated as a cause of biased sex ratios at birth. We used gonadal anatomy, or occasionally sexual dimorphism at maturity, to sex offspring unequivocally (hemipenes are present in both sexes at birth). Among lab-gestated clutches from previous studies examining other aspects of gestation, we found a highly significant bias toward same-sex clutches in twin pregnancies (19 of 21 clutches; $p<0.001$), and a significant bias towards females overall when singleton clutches were included (37f: 17m; $p<0.01$). Neither female body condition nor estimated gestation length (a possible surrogate for thermal conditions) influenced clutch sex ratios. Mothers did not obviously allocate more energy to offspring of different sex (offspring mass, with maternal mass as a covariate, did not differ between males and females). Further work is exploring experimentally the effect of gestational temperature, but colony sex ratio should also be considered. We suggest that a bias toward females may be adaptive in a species with prolonged gestation in which females do not always reproduce each year. Our results, for a gecko in which viviparity has evolved independently of other major lineages, strengthen reports of diverse sex determination patterns among reptiles.

043

AMPHIBIAN GENOME CRYOBANKING – SUCCESS IN SPERM CRYOPRESERVATION BUT THE BLOCK TO EMBRYO CRYOPRESERVATION REMAINS.

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The technologies of Assisted Reproduction (ART) have the potential to assist in the management of the amphibian extinction crisis by improving the cost-effectiveness and efficiency of captive husbandry and genetic management of small populations, as well as providing cryo-storage systems that insure against extinction. Sperm and embryo cryopreservation are two important aspects of amphibian ART. Of these, sperm cryopreservation has been successful in a number of species, but recovery of cryopreserved embryos remains elusive. We report here further investigations of these approaches. Experiments with *B. marinus* sperm (utilizing this species as a general amphibian model) found higher recovery at intermediate (1-5^{o}C/min) than slow (< 1 ^{o}C/min) freezing rates, and no recovery at the highest rate (vitrification). *B. marinus* sperm showed susceptibility to cold shock (a rapid reduction in temperature from ambient to just above 0^{o}C), but this was absent or less pronounced in sperm of 4 native species investigated. *B. marinus* sperm were also susceptible to damage from centrifugation with the level of damage positively correlated to centrifugation force. Use of cryopreservation protocols developed for *B. marinus* in two native hylids (*Litoria revelata* and *Lit. latopalmata*) and two native myobatrachids (*Pseudophryne coriacea* and *Uperoleia fusca*) resulted in recovery of motile sperm from all species. However, recovery of sperm viability (as assessed by live/dead stains) was lower in the myobatrachid species; recovery in the hylids was similar to *B. marinus*. Attempts to cryopreserve whole embryos of *Limnodynastes peronii* and *Crinia signifera* were unsuccessful. Embryos of these species showed some tolerance to cryoprotectants at temperatures above 0^{o}C (tested because extended exposure pre-freezing may improve cyroprotectant penetration), but embryo survival was reduced as a function of both exposure time and concentration. These data together further reinforce the view that amphibian sperm cryopreservation is feasible across a range of taxa, but that successful whole amphibian embryo cryopreservation continues to be elusive.

044

PRESERVATION OF SPERMATOZOA FROM DASYURID MARSUPIALS

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Dasyurids are carnivorous marsupials which play fundamental roles in Australia's ecosystems. The dasyurid family is diverse containing quolls, Tasmanian devils and dunnarts but all share the common feature of large (300µm), asymmetric and fragile spermatozoa lacking in nuclear or acrosomal disulphide stabilization. There are currently no established protocols for preservation of dasyurid spermatozoa however reports of limited motility following cryopreservation with 15% egg yolk and 8% glycerol exist (1). This study examines preservation of dasyurid spermatozoa using the fat-tailed dunnart (*Sminthopsis crassicaudata*) as an experimental model. In cryopreservation experiments, spermatozoa from twelve animals (n=12) frozen in media enriched with 15% egg yolk were not viable when assessed with SYBR14 and propidium iodide and had abnormal sickle shaped sperm heads. These adverse effects were also apparent prior to freezing, indicating a negative effect of 15% egg yolk in dasyurids. Cryopreservation of dunnart sperm in yolk free media (tris-citrate-fructose buffer with a final concentration of 40% glycerol) produced a low percentage of viable but non-motile sperm for up to 40 minutes post-thaw (n=5). At lower glycerol concentrations sperm were not viable, and duplicate counts of 100 sperm following Bryan's staining indicated significant acrosomal loss in preparations containing 8% ($P<0.001$, n=5) and 16% ($P<0.01$, n=5) but not 20% glycerol (n=5). As we are investigating intracytoplasmic sperm injection in dasyurids alternative preservation techniques were also examined. Drying spermatozoa under a stream of nitrogen (n=5) resulted in complete nuclear decondensation and head loss upon rehydration. Freeze drying (n=5) was a more promising technique with only 20% loss of acrosomal integrity and no significant head loss. This study provides insights into methods of preservation appropriate for use in dasyurid species. Very high glycerol concentrations, normally toxic to eutherians and the less susceptible marsupials, were required to retain viability. The reason for the apparent detrimental effects of egg yolk is not clear, and as this finding differs from previous reports it warrants further investigation.

(1) Taggart DA, Leigh CM, Steele VR, Breed WG, Temple-Smith PD, Phelan J (1996) Reprod Fert Dev 8: 673-679

045

CLONING AND FUNCTIONAL ANALYSIS OF PROACROSIN FROM A MARSUPIAL, THE TAMMAR WALLABY (MACROPUS EUGENII)

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Our understanding of the molecular basis of the mammalian fertilization process is based almost entirely on studies in eutherian species. Yet the role and relative importance of individual sperm proteins in mediating sperm-egg interactions remains controversial. Proacrosin is the zymogen of β-acrosin, a sperm acrosomal protease with several proposed roles in the mammalian fertilization process including secondary zona binding, dispersal of acrosomal contents and zona pellucida digestion. This study reports the cloning and functional analysis of proacrosin for the first time from a marsupial. Thus it is of interest not only to the study of marsupial fertilization mechanisms, but also in providing comparative insights into the role of proacrosin in eutherian fertilization. The primary structure of tammar wallaby preproacrosin was determined from the full-length nucleotide sequence isolated from a

testis cDNA library. The deduced 431 amino acid (aa) sequence of wallaby preproacrosin contained an 18 aa signal sequence, 20 aa light chain, a heavy chain and proline rich C-terminus which may consist of 303 and 90 aa respectively. Wallaby preproacrosin demonstrated homology with eutherian and ascidian preproacrosin sequences as well as other serine proteases. Many structural and functional features identified in eutherian proacrosin sequences were conserved in wallaby proacrosin. The substrate recognition site, catalytic triad and IVGG motif characteristic of serine proteases were present in the wallaby acrosin heavy chain. Complete conservation of the twelve cysteine residues involved in disulfide bond formation, and subsequent protein folding, allowed for three dimensional modeling of the tertiary structure. This model indicated that wallaby proacrosin is structurally similar to the boar proacrosin crystal structure. There are however marked differences in the composition, location and spatial conformation of the basic amino acid residues likely to mediate zona-binding in wallaby proacrosin. The structural features of wallaby proacrosin, together with localization and zona-binding studies suggest that it is likely to play an important role in the marsupial fertilization process.

046

PATTERNING FACTORS IN THE DEVELOPING GONAD OF THE MARSUPIAL

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Sex determination in mammals is dependent upon the presence or absence of the *SRY* (sex-determining region on the Y) gene on the Y-chromosome to activate a wide range of transcription and patterning factors that are essential for the differentiation of the testis. FGF9 (fibroblast growth factor 9), DHH (desert hedgehog) and BMP4 (bone morphogenetic protein 4) play important roles in the patterning of the gonads during early development and are critical for the differentiation and specification of specific cell types in the mouse testis. BMP4 is also essential for germ cell differentiation in the ovary. However, the function of these patterning factors has not yet been examined in any marsupial. This study characterized these factors and examined their function during marsupial sexual differentiation. Tammar *FGF9* was cloned using PCR and had over 90% homology with human *FGF9*. *DHH* and *BMP4* were characterized in the tammar in previous studies, and both had greater than 80% homology with their respective human homologues. *FGF9* was expressed in the testis and ovary at two days before birth up to day 15 postpartum. Developing marsupial gonads at day of birth (n=7) were cultured in DMEM for 5 days. The inhibitors aphidicolin (10µg/ml), forskolin (20mM) and noggin (1µg/ml), specific for each of the signaling pathways of FGF9, DHH and BMP4, were added to the cultures, and DMSO or PBS was added to the controls. Morphology of gonads were analysed after culture using standard histological staining methods and testicular and ovarian differentiation markers SOX9 and AMH were analyzed using immunocytochemisty. The results suggest that FGFs, HHs and BMPs in the marsupial have similar roles to those in other mammals and are critical for normal gonadal differentiation in the tammar.

047

EXPRESSION OF EGF AND EGF-R IN THE ENDOMETRIUM DURING ENTRY INTO AND REACTIVATION FROM EMBRYONIC DIAPAUSE IN THE TAMMAR WALLABY, *MACROPUS EUGENII.*

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Embryonic diapause is widespread amongst mammals, but is especially common in the kangaroos and wallabies. In the tammar, *Macropus eugenii*, the sequence of endocrine events leading to diapause and reactivation are well defined (Renfree and Shaw 2000). The ovarian hormones exert their effects on the blastocyst by alterations in the endometrium and its secretions, but the molecular interactions between the endometrium and blastocyst are unknown. There is increasing evidence for the involvement of leukaemia inhibitory factor (LIF) but the epidermal growth factor (EGF) family of growth factors are also likely to be involved. This study examined the expression of EGF and epidermal growth factor receptor (EGF-R) in the tammar endometrium at entry into and reactivation from diapause. EGF and its receptor were cloned and their sequences were strongly conserved with EGF and EGF-R of man and mouse (hEGF 79%, hEGF-R 78%, mEGF 75%, mEGF-R 75% homology). *EGF* and *EGF-R* expression was assessed by RT-PCR and both genes were expressed in the endometrium at all stages examined. Quantitative PCR studies are in progress to determine if there is any change in levels of expression between the stages examined. A novel splice variant of EGF has been identified which lacks exon 10 however the significance of this is unknown. *In situ* and immunohistochemical studies are in progress to establish if there is any change in cellular location of EGF between stromal and epithelial cells as seen in human endometrium during the menstrual cycle and in gestational decidua (Hofmann *et al.* 1991). These results suggest that diapause in the tammar is controlled by more than one growth factor.

(1) Hofmann GE, Scott RT, Jr, Bergh PA, Deligdisch L (1991) Immunohistochemical localization of epidermal growth factor in human endometrium, decidua, and placenta. Journal of Clinical Endocrinology and M

(2) Renfree MB, Shaw G (2000) Diapause. Annu Rev Physiol 62, 353-375.

048

THE EFFECTS OF OESTROGEN ON SEXUAL DIFFERENTIATION IN A MARSUPIAL, MACROPUS EUGENII

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The interaction of genetic and hormonal pathways is critical for the establishment of sex in mammals. Sexual differentiation in marsupials such as the tammar wallaby, Macropus eugenii, occurs after birth. In males, testis cords form by day 2 post partum (pp) and androgen synthesis begins at about the same time, while in female ovaries do not develop cortical and medullary regions until around day 8pp (Renfree et al 1992; 1996). Birth normally occurs after an active gestation period of 26.5 days, but 'premature' births can occur on day 25 of gestation. At this age, gonads of both sexes are undifferentiated. Daily oral administration of oestradiol-17α to male pouch young born after 25 days of gestation induces formation of an ovarian-like structure in the testes by day 50 after birth (Coveney et al., 2001; Renfree et al., 2001). When gonads from male fetuses of 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. Control female gonads were unchanged by addition of extra oestrogen. Real-time PCR was used to determine and compare expression of key sex determination genes during this sex reversal. This culture system therefore provides an excellent mammalian model to examine the molecular control of ovarian differentiation.

(1) Coveney D et al., (2001) Biol of Reprod 65: 613-621 Estrogen-Induced Gonadal Sex Reversal in the Tammar Wallaby
(2) Renfree MB et al., (1992) J Zoology 226: 165-173 The role of genes and hormones in marsupial sexual differentiation
(3) Renfree MB et al., (1996) Anat. Embryol 194: 111-134 Sexual Differentiation of the uroenital system of the fetal and neonatal Tammar wallaby.
(4) Renfree MB., (2001) Reproduction Fertility and Development 13: 231-240 The Influence of Estrogen on the Developing Male Marsupial.

049

INSIGHTS INTO REPRODUCTIVE BIOLOGY

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Abstract not provided

050

ANDROGEN DEFICIENCY

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Androgen deficiency (AD) affects 1 in 200 men under 60 years. Symptoms and signs may be subtle or non-specific and many cases remain undiagnosed. When identified by clinical features and confirmatory endocrine testing (ESA Guidelines, 2002), AD requires treatment irrespective of age. Primary testicular disease (low testosterone [T], high LH) may present with infertility so that co-existent AD must be considered in that setting. Klinefelter's syndrome (47XXY) is the commonest cause of AD yet 70% of cases remain undiagnosed. Secondary AD (low T, low LH) results from hypothalamo-pituitary disorders and may be accompanied by other hormonal deficiencies. The total T level is the key clinical measure; the timing and number of samples, the assay method and reference interval effect its interpretation. Improved clinical outcomes based on free T levels have not been proven.

The definition and prevalence of AD in association with ageing *per se* is contentious. Symptoms consistent with, but not diagnostic of, AD increase with age while serum T levels fall around 1-2% annually leading to the proposition that older men may derive gain (at acceptable risk) from T treatment. However concomitant illnesses, particularly obesity, may confound evaluation as they are associated with lower serum T levels and similar symptoms: clinical outcomes may be improved by attention to specific health problems, lifestyle and relationship issues. Placebo-controlled RCTs of testosterone therapy in older men with low-normal T levels show only modest improvement in body composition, inconsistent effects on physical function and QOL, and all lack long term safety data. Current research studies are exploring the utility of TRx in metabolic syndrome, 'PDE5 refractory' erectile dysfunction with low-normal T levels, and as an anabolic agent to improve nutrition, strength & function in chronic wasting diseases.

Testosterone therapy (TRx) is effective in restoring normal sexual, bone and muscle health, and quality of life (QOL), and potentially in normalising cardiovascular risk. Transdermal systems, crystalline T implants and injectable T ester formulations acceptable serum T profiles. The new long acting T undecanoate intramuscular formulation provides physiological T levels with a dose interval of 10-14 weeks. Absolute contraindications to TRx are recognised (esp. prostate or breast cancer) and caution indicated in other settings (e.g. severe sleep apnoea, BPH with significant obstructive symptoms). Age-appropriate health monitoring is required along with attention to polycythemia, sleep disturbance, and monitoring of prevalent diseases in older men such as prostate and cardiovascular disease.

051

MATERNAL RESPONSES TO DEPORTED TROPHOBLASTS

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Viviparous pregnancy presents a unique challenge to the maternal organism with 50% of the fetal/placental genetic material being derived from an immunologically distinct individual, ie, the father. Yet this graft normally survives during pregnancy without the need for immunosuppressive drugs. Preeclampsia, one of the most common diseases of pregnancy appears to have an immunological component. In preeclampsia maternal blood pressure is elevated and perfusion of maternal organs, as well as the placenta is reduced leading to fetal mortality and morbidity and serious maternal complications, including death. What causes preeclampsia is unknown and there are both fetal and maternal components to the disease process. However, it is clear that the initial triggering factor is derived from placental trophoblasts since preeclampsia only occurs in pregnancy or in women with trophoblastic tumours. The definitive cure for preeclampsia is delivery of the placenta, yet preeclampsia can strike several days after delivery, suggesting that the placental triggering factor persists in the maternal organism for several days. Syncytial knots are multinucleated fragments of the syncytiotrophoblast which are shed into the maternal blood as they die. In normal pregnancy syncytial knots are produced as a result of apoptosis but this death process may be altered in preeclampsia. Syncytial knots are deported in the blood and become trapped in maternal pulmonary capillaries where they may remain for up to 14 days before being cleared and thus are candidates for the preeclampsia triggering factor. We have investigated the mechanisms by which syncytial knots are cleared and the effects of the cell death process that the syncytial knots undergo on both the immune and vascular systems. We have shown that dead trophoblasts are phagocytosed by macrophages and endothelial cells. Phagocytosis of apoptotic trophoblasts induced synthesis of IL-10 and the immunosuppressive enzyme indoleamine 2,3 dioxygenase (IDO) while phagocytosis of necrotic trophoblasts activated endothelial cells, increased monocyte adhesion, and promoted secretion of factors that promoted the activation of additional endothelial cells.

052

MALE SEMINAL FLUID REGULATION OF FEMALE TRACT RECEPTIVITY FOR EMBRYO IMPLANTATION

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Attenuation in the cellular structure and function of the endometrial tissue confers transient maternal receptivity to embryo implantation during early pregnancy. Embryo attachment and trophoblast cell invasion can only occur when the biochemistry of the uterine epithelial cell surface allows close apposition and then adhesion between the blastocyst and the luminal surface. Fluctuating expression in adhesion and anti-adhesion molecules provides a barrier until the window of implantation when the embryo firstly becomes anchored in close proximity to the epithelium, and then initiates trophoblast invasion to form the firm connections vital for further development. Although these changes are largely directed by ovarian steroid hormones, recent evidence suggests that factors derived in male seminal fluid may contribute to generating uterine receptivity in mammalian species where uterine intromission occurs. Seminal fluid delivered to the female tract at insemination elicits an inflammatory response characterised by elevated cytokine and chemokine expression and recruitment of macrophages and other inflammatory leukocytes. When seminal fluid signalling is ablated at the outset of pregnancy, implantation is compromised, placental development is altered, and fetal and post-natal growth is adversely affected. We have hypothesised that macrophages recruited in response to seminal fluid signalling have roles in the tissue remodelling and acquisition of epithelial cell adhesiveness which underpin the receptive state. Experiments to evaluate the role of seminal fluid-regulated macrophages have shown key roles for macrophage-derived signals in regulating expression of mRNAs encoding various adhesion and anti-adhesion molecules implicated in implantation success, including fucosyltransferases (FUT)-1, FUT2 and FUT4 and mucins (MUC)-1 and MUC4. Our data demonstrate that exposure to male seminal fluid can regulate endometrial epithelial phenotype in preparation for embryo implantation and that the effect is mediated via the agency of factors secreted from macrophages recruited during the inflammatory response to insemination. Dysregulation in the changes underpinning uterine receptivity may impair early placental morphogenesis and cause the compromised fetal development evident when male seminal fluid signalling is disrupted.

053

IS THERE A MATERNAL INFLUENCE ON SEX ALLOCATION IN MAMMALS?

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Evidence from evolutionary biology suggests that the allocation of the sex of the offspring in mammals may not, after all, be a matter of chance. After briefly summarizing this evidence I will show how some of its features have led me to think that mammalian mothers could have some influence on the determination of the sex of their offspring. In general this pathway leads from the behavioural evidence (dominance) to female testosterone, which in turn may provide a link to a proximate mechanism influencing sex allocation. I explain this hypothesized proximate mechanism and then present recent data that appear to support it. I also suggest a way in which these findings may be rendered compatible with sex ratio data from pregnancies following fertilization with sex-sorted sperm.

054

EPIGENETIC MODIFIERS SHOW PATERNAL EFFECTS IN THE MOUSE

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There is increasing evidence that epigenetic information can be inherited across generations in mammals, despite extensive reprogramming both in the gametes and the early developing embryo. One corollary to this is that disruption of the establishment of epigenetic state in the gametes of a parent, as a result of heterozygosity for mutations in genes involved in reprogramming, could affect the phenotype of offspring that do not inherit the mutant allele. Here we show that such effects do occur following paternal inheritance in the mouse. We detect changes to transcription and chromosome ploidy in adult animals. Paternal effects of this type have not been reported previously in mammals and suggest that the untransmitted genotype of male parents can influence the phenotype of their offspring.

055

IDENTIFYING NOVEL CONTRACEPTIVE TARGETS: TRANSCRIPTIONAL PROFILING OF SPECIFIC STAGES OF RAT SPERMATOGENESIS DURING HORMONE SUPPRESSION

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In vivo suppression of testosterone (T) and FSH to inhibit sperm production is a promising approach to male contraception. The mid spermatogenic stages (VII and VIII in the rat) are particularly responsive to hormone suppression, and release of sperm from Sertoli cells (spermiation) during these stages is a major target for hormone-based contraceptives. The current study aimed to identify hormone-responsive genes during mid-spermatogenesis and gain insights into which genes may be involved in spermiation.

Acute FSH and androgen suppression was achieved in adult male SD rats using steroid implants, daily injections of an FSH antibody and an androgen receptor antagonist (flutamide) for 4 days, whereas control rats received vehicle injections but no implants (n=6/group). Stereological analysis indicated hormone suppression produced minor changes in pachytene spermatocyte numbers (<30% reduction), no changes in round spermatid numbers yet profound spermiation failure (% spermatids failing to spermiate was 90.4±27.3 in hormone-suppressed rats vs 2.7±1.5 in controls, mean±SEM, $p<0.05$). Stage VII and VIII tubules were dissected under transillumination prior to RNA extraction. Total RNA (concentration, purity and integrity assessed by Bioanalyser, 260:280nm ratio and agarose gel) underwent one round of amplification to yield cRNA. 15 m g biotin-cRNA was hybridised to Affymetrix GeneChip Rat Genome 230 2.0 arrays, containing 31,000 probe sets to analyse >28,000 rat genes. Expressionist™ Analyst software was used to identify genes showing a statistical difference or >4 fold difference between stage VII and VIII tubules in normal rats, and between control and hormone-suppressed rats in each stage.

The results suggested that the transition between stages VII and VIII in normal rats is associated with transcriptional repression (153 down-regulated genes vs 34 up-regulated genes). During acute hormone suppression 77 & 67 genes were up-regulated and 33 & 38 genes down-regulated in stages VII and VIII, respectively, and transcriptional repression in stage VIII seemed to be impaired. Genes showing changes in response to hormone suppression included those with putative roles in adhesion dynamics (eg. testin, galectin, integrin, catenin), signalling (eg. adenylyl cyclase 2), cell cycle (eg. retinoblastoma-like 2) and phosphorylation events (eg. SNF-related kinase).

These results provide insights into the mechanisms by which hormones influence spermatogenesis and further studies are underway to elucidate the function of particular genes in relation to spermatogenesis and spermiation.

056

AN ACUTE MACROPHAGE DEPLETION MODEL REVEALS A ROLE FOR MACROPHAGES IN REGULATION OF TESTICULAR STEROIDOGENESIS IN VIVO

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Macrophages are versatile cells with roles in the inductive phase of immune responses, phagocytosis, tissue remodelling and production of regulatory cytokines. Knockout, in vitro and descriptive studies have indicated macrophages have many diverse roles in both male and female reproductive biology (1,2). Mice carrying a monkey diphtheria receptor (DTR) transgene driven by the macrophage specific promoter CD11b can be acutely depleted of macrophages in a number of different tissues by administration of

diphtheria toxin (DT) (3). These mice offer a unique opportunity to study the role of macrophages in precise reproductive events. We have used this model initially to investigate the role of macrophages in regulation of testicular steroidogenesis. 24 hours following DT administration, testicular macrophages were depleted from CD11b-DTR mice and remained absent for at least 24 hours. In female CD11b-DTR mice, a similar DT administration protocol was found to cause macrophage depletion from the ovary and uterus. Macrophage depletion in males corresponded with significant increases in testis mRNA encoding the steroidogenic regulatory enzymes StAR (3-fold), P450scc (2.5-fold) and P450c17 (2-fold) ($p<0.05$), while mRNA expression of 3bHSD was unaltered. However there was no change observed in intratesticular or serum testosterone levels, recovered 24 hours after DT administration in unprimed mice, or mice primed by intraperitoneal treatment with LH/hCG. These results indicate that macrophages have a predominantly inhibitory role in regulation of testicular steroidogenesis, however their role is insufficient to affect the physiological concentration of testosterone in the short term. This model has potential for greater analysis of the impact of macrophages in specific reproductive events.

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057

TRANSIENT *IN UTERO* EXPOSURE TO THE ENDOCRINE DISRUPTOR VINCLOZOLIN INDUCES INFLAMMATION AND ATROPHY IN THE POST- BUT NOT PRE-PUBERTAL PROSTATE

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Fetal exposure to the anti-androgenic fungicide Vinclozolin has been shown to have adverse effects on male reproductive tract development. Recent studies have suggested the adult prostate may also be altered by exposure to Vinclozolin. However, it is not clear whether the reported prostatic pathology occurs earlier in development, nor is it clear whether growth regulatory pathways may be affected. Therefore, the aim of this study was to characterise the effects of transient *in utero* exposure to Vinclozolin on the pre- and post-pubertal rodent prostate gland.

Fetal rats were exposed to Vinclozolin (100mg.kg bw) or corn oil vehicle control (2.5ml.kg bw) *i n utero* for 6 days via oral administration to pregnant dams. Male pups were aged to 4 or 8 weeks (pre-pubertal; post-pubertal respectively) before tissue was collected for analysis. At 4 weeks of age *in utero* exposure to Vinclozolin resulted in no significant developmental or morphological abnormalities compared to control animals. Prostates of Vinclozolin-treated post-pubertal animals displayed apparent epithelial atrophy which was confirmed by subsequent stereological analysis and immunohistochemistry revealed a significant increase in the percentage of basal cells within epithelia of atrophic glands. Analysis of hormone receptor expression revealed reduced epithelial and increased stromal AR expression in Vinclozolin-treated tissues, although no differences in estrogen receptor alpha or beta expression were observed. An apparent increase in inflammatory cells was observed in Vinclozolin treated tissues and preliminary studies suggest a link with up regulation in the NF k B signaling pathway.

Overall, this study demonstrates that transient *in utero* exposure to an anti-androgenic chemical has the potential to disrupt normal prostate development and induce an inflammatory response which only becomes identifiable in post-pubertal animals implying aberrant androgenic response. This work is of particular significance as there is increasing literature suggesting a link between chronic inflammation and prostate cancer.

058

GENETIC AND FUNCTIONAL ANALYSIS OF PACRG, A NOVEL PROTEIN ASSOCIATED WITH HUMAN MALE INFERTILITY

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Male infertility is a major medical problem affecting approximately 15% of couples in the western world. Significantly, 30% of infertility cases presenting with abnormal sperm production are idiopathic, suggesting additional 'infertility' genes remain to be identified. The function of recently identified, Parkin Co-Regulated Gene (PACRG) is unknown; however Pacrg-mediated correction of the flagella defect in the quaking viable (*qkv*) mouse suggests a role in spermiogenesis (Lorenzetti et al., 2004). Using *in situ hybridization*, western blot analysis and immunocytochemisty we have shown Pacrg is highly expressed in mouse testes from 3 weeks of age through to adulthood. Pacrg was detected in the mid- and principal pieces of the tail of isolated spermatozoa, consistent with a role for Pacrg in flagella formation and function. To characterise the role of Pacrg in spermatogenesis, we generated transgenic mice over-expressing *Pacrg* under control of the endogenous Pacrg (END-Pacrg) promoter. The END-Pacrg construct rescued the *qkv* infertility phenotype, determined by daily sperm production, sperm morphology and fecundity analysis.

Given the evidence implicating Pacrg in mouse spermatogenesis, we investigated the potential contribution of PACRG to human male fertility. Sequence analysis of the PACRG promoter region and 5 coding exons was conducted on a preliminary cohort of 46 infertile men diagnosed with azoospermia or severe oligospermia, these phenotypes are analogous to the *qkv* infertility phenotype. No coding sequence alterations were detected. However, three non-coding sequence alterations were identified, two in the 5' untranslated region (rs9347683 and -227A>G, (West et al., 2002) and a novel SNP 11bp upstream of exon 2 (IVS1+85746T>C). An

association study was performed in a case-control cohort of 206 infertile men diagnosed with azoospermia and 156 fertile controls. No association was identified for either -227A>G or IVS1+85746T>C variants. However, a statistically significant association between the minor allele of rs9347683 and male infertility was observed (P= 0.009 Fisher Exact χ^2 test; OR=1.7, 95% CI=1.01-2.59), suggesting genetic variation of PACRG may represent a risk factor for human male infertility.

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059

IDENTIFYING A ROLE FOR INFLAMMATORY INTERMEDIATES IN GERM CELL-SERTOLI CELL COMMUNICATION

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Sertoli cells respond directly to a variety of inflammatory stimuli, in turn producing regulatory cytokines, such as interleukin-1α (IL1α), IL6 and activin A, which exert direct effects on germ cell proliferation and development. These mechanisms are crucial to testicular responses to infection and inflammation. However, emerging data indicate that spermatogenic cells themselves produce inflammatory molecules, including tumour necrosis factor α (TNFα) and endogenous toll-like receptor (TLR) ligands, such as high mobility group box chromosomal protein 1. These observations suggest a role for inflammatory pathways in control of normal functions within the seminiferous epithelium. Sertoli cells were isolated from 20-day old rat testes and cultured for between 3 and 48 h. Expression of TLR components and cytokines were measured by quantitative RT-PCR, ELISA and/or Western blot. Ligands for TLR4 (purified lipopolysaccharide; LPS) and TLR2 (Pam3Cys) stimulated Sertoli cell production of IL1α, IL6 and activin A. High levels of expression of TLR4 and MD2, the core proteins of the LPS receptor complex, were found in the Sertoli cells. Expression of the TLR4 receptor co-factor, CD14, and TLR2 were relatively lower at the mRNA level. The production of activin A by Sertoli cells stimulated by LPS and TNFα, but also by IL1α, was blocked by inhibitors of the stress-responsive MAP kinase pathway (p38 MAP kinase and Jnk), but not by the NFκB inhibitors, SN50 and MG132. Testosterone and cAMP also inhibited this production in a synergistic manner. The expression of functional TLRs on the Sertoli cells and responsiveness to TLR ligands and TNFα, as well as the Sertoli cell-derived cytokine IL1α, suggest the potential for novel mechanisms of signalling between the spermatogenic cells and their supporting cells involving traditionally "inflammatory" pathways. It is hypothesised that signalling via these pathways may facilitate localised communication within the normal seminiferous epithelium in conjunction with overall hormonal control of spermatogenesis, and disruption of this signalling during inflammation or infection may contribute to testicular failure in various pathological conditions.

060

MALE MICE WITH ANDROGEN RECEPTOR DISRUPTION TARGETING SEX ACCESSORY ORGANS ARE SUBFERTILE

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Androgen action on sex accessory glands influences rodent fertility but the mechanisms remain unclear and investigation is difficult without the ability to restrict androgen action or inactivation to specific tissues. We found that PEARKO males which features androgen receptor (AR) inactivation restricted to the prostate epithelium together with epididymis and seminal vesicles[1] are subfertile compared to littermate controls (5/15 vs 11/11 fertile, $p<0.05$). Detailed analysis of PEARKO males at 8 week of age revealed significantly decreased weight of prostate lobes (36-80% of control), seminal vesicles (55%) and epididymis (78%) while serum testosterone, testis weight and total homogenization-resistant sperm head count in testis and cauda epidiymis were unaltered compared with littermate controls. Copulatory plugs were detected four hours post mating, but the plugs produced by PEARKO males were smaller (3.9±0.5 vs 18.1±0.5 mg, $p<0.05$), located deeper in female reproductive tract and had softer consistency than controls. Quantitative fertilising ability of epididymal sperm *in vitro* was normal in PEARKO males but after natural mating fewer fertilized oocytes were flushed from the oviducts of females (8/37 vs 50/71 fertilized, $p<0.05$). This data suggest that sperm formed in mice with impaired androgen action in accessory glands and epididymis are quantitatively normal in number and function (*in vitro* fertilising ability) but that subfertility reflects other functions extrinsic to sperm that determine fertility *in vivo*. Further studies to identify the tissue localisation and nature of the functional defect in male fertility of males with impaired androgen action in sex accessory glands are warranted. These findings may have implications for better understanding of mechanisms of idiopathic male infertility and for novel tissue targetted hormonal male contracetives.

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061

EFFECT OF GONADOTROPHIN SUPPRESSION ON TESTICULAR TIGHT JUNCTIONS

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Sertoli cell tight junctions (TJs) are essential for spermatogenesis as they divide the epithelium into basal and adluminal compartments. Evidence suggests that TJs are regulated by gonadotrophins *in vitro* and *in vivo.* The aim of this study was to investigate the effect of gonadotrophin suppression and selective short-term replacement of FSH and/or testosterone on TJ proteins (occludin, claudin-11) and TJ function in an adult rat model.

Following gonadotrophin suppression (acyline injection, weekly, 7 weeks), adult rats (n = 10/group) received short-term hormone replacement (daily, 7 days) of hCG alone (2.5IU/kg) (to stimulate testicular testosterone production), hFSH alone (25IU/kg) or hCG and hFSH. Testes were then sampled for real time RT-PCR and immunohistochemistry of TJ proteins, and a qualitative functional assessment of tight junction permeability was performed using a biotin tracer localisation technique.

Acyline reduced testis weights to 19% ($p < 0.001$) of control, which then increased to 23.5-27.0% ($p < 0.01$) in the hormone treated groups (*ns* between groups). In controls, biotin tracer localised into the basal compartment, but did not pass beyond Sertoli cell TJs, as shown by localisation with occludin. After acyline treatment, both basal and adluminal compartments of tubules were permeable to biotin and occludin staining was no longer visible. Occludin re-localised to the TJ in hCG ± FSH-treated rats, but not in rats treated with FSH alone. In each treatment group, morphology was partially restored as shown by the reappearance of tubular lumens, but most tubules remained permeable to biotin, even in the presence of occludin staining. These results suggest i) that Sertoli cell TJs in the adult rat are functionally regulated by gonadotrophins, ii) that localisation of occludin at TJs requires at least hCG (testosterone) but apparently not FSH, and iii) that TJ protein (occludin) localisation at Sertoli cell TJs occurs prior to restoration of TJ function. It is concluded that Sertoli cell TJs are functionally regulated by gonadotrophins in this GnRH antagonist-treated adult rat model. This study has potential significance in understanding testicular mechanisms of hormone action in men undergoing hormonal contraception.

062

THE ROLE OF GAMETOGENETIN (GGN) IN SPERMATOGENESIS AND PRE-IMPLANTATION-EMBRYO DEVELOPMENT

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Cysteine-rich secretory protein 2 (CRIPS2) is a testis-enriched protein localised to the sperm acrosome and tail. CRISP2 has been proposed to play a critical role in spermatogenesis and male fertility, although the precise function(s) of CRISP2 remain to be determined. Recent data has shown that the CRISP domain of CRISP2 has the ability to regulate Ca^{2+} flow through ryanodine receptors (RyR) and to bind to the mitogen-activated protein kinase kinase kinase 11 (MAP3K11). To fu rther define the biochemical pathways within which CRISP2 is involved, we screened an adult mouse testis cDNA library to identify CRISP2 interacting partners. One of the most frequently identified CRISP2 binding protein was gametogenetin 1 (GGN1). Interactions occurred between the ion channel regulatory region (ICR) within the CRISP2 CRISP domain and the carboxyl-most 158 amino acids of GGN1. Further, we have shown that GGN1 is a testis-enriched mRNA and that protein is first expressed in late pachytene spermatocytes and up-regulated in round spermatids before being incorporated into the growing principal piece of the sperm tail specifically the longitudinal columns of the fibrous sheath in a position consistent with the expression of CRISP2 protein. These data along with data on RyR and MAP3K11 binding define the CRISP2 CRISP domain as a protein interaction motif and suggest a role for the GGN1-CRISP2 complex in sperm tail assembly or motility. To further define the role of *Ggn* we generated *Ggn* knockout mice. Surprisingly, the lack of *Ggn* expression resulted in embryonic lethality in the homozygous knockout mice prior to the 1-cell stage of development as determined by *in vitro* fertilization. Using Western blot analysis, we have shown that GGN1 is found in the ovulated oocyte. Our findings indicate that GGN plays a critical role in the earliest stages of embryonic development, but in the adult interacts with CRISP2 during sperm formation.

063

SUCCESSFUL TREATMENT OF INAPPROPRIATE HYPORENINAEMIC HYPOALDOSTERONISM IN CEREBRAL SALT WASTING SECONDARY TO BILATERAL SUBDURAL HAEMATOMAS WITH FLUDROCORTISONE

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Hyponatraemia is a common complication following cerebral trauma. It has most commonly been attributed to the syndrome of inappropriate secretion of anti-diuretic hormone (SIADH). Cerebral salt wasting (CSW), with release of anti-diuretic hormone as a result of volume depletion from cerebrally mediated urinary salt wasting can lead to a similar clinical picture, for which the pathophysiology is poorly understood and treatment not well defined. We describe a 75 year old man who developed CSW secondary

to bilateral subdural haematomas, with inappropriate hyporeninaemic hypoaldosteronism. He was dependent on hypertonic saline infusion to maintain eunatraemia. The case study highlights the diagnostic challenge of the differentiation between SIADH and CSW, but more importantly the management dilemma in refractory hyponatraemia following cerebral trauma. Fludrocortisone has been used to prevent volume depletion and ischaemia in patients with subarachnoid haemorrhage, but its use in other cerebral pathologies are poorly studied, with only scatted case reports in the paediatric literature. This is the first report in adult endocrinology describing the changes in serum and urinary biochemistry in a patient with inappropriate hyporeninaemic hypoaldosteronism and the response to fludrocortisone, suggesting a role of mineralocorticoid therapy in selected adult patients with refractory hyponatraemia following cerebral trauma.

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064

CARBIMAZOLE-INDUCED AGRANULOCYTOSIS

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Background: Agranulocytosis is an uncommon but potentially fatal complication of antithyroid drugs. We describe the management of a patient with febrile neutropenia who presented two weeks following commencement of carbimazole for Graves' disease

A 30 yo lady presented with a one month history of hyperthyroid symptoms including weight loss of three kgs and proximal myopathy. She had developed pyoderma gangrenosum at the site of a cesarean section scar eleven months prior. This required surgical debridement and treatment with prednisolone up to 60mg for four months and cyclosporin 125mg bd for nine months. Cyclosporin was ceased three weeks prior to her presentation with thyrotoxicosis. Her TSH was 0.02, fT4 was 70.7 and fT3 was 41.8 .Initial investigations confirmed the clinical suspicion of Graves' disease. She was commenced on carbimazole 10mg tds, propranolol 20mg bd, and received verbal and written instructions regarding the risk of agranulocytosis. At her two week follow-up appointment, she complained of a severe sore throat and was found to be febrile 37.9 ^{o}C. Her Hb was 101 g/L, total white cell count 1.7 x 10^9/L and a neutrophil count of 0.2 x 10^9/L, platelets were 404 x 10^9/L. She was admitted to the hospital for and given broad spectrum iv antibiotics and GCSF 263 ug sc/d for six days. Repeat thyroid function tests were within normal limits, thyroid uptake scan showed 8% uptake. Because of the potential for pyoderma recurrence in her thyroidectomy scar, she opted against thyroidectomy. She was given 10 mCi 131 Iodine. Neutrophil count normalised day seven and the patient was discharged. She remained euthyroid at follow up eight weeks later.

The following issues will be discussed :

- incidence of agranulocytosis in patients who are on antithyroid drugs
- role of GCSF
- options for the subsequent management of thyrotoxicosis
- link between history of pyoderma gangrenosum in this patient and carbimazole associated agranulocytosis

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065

GRANULOMATOUS DISEASE AS A CAUSE OF SEVERE HYPERCALCAEMIA.

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Case report: A 65 year old man was referred for investigation of severe hypercalcaemia. He had a past medical history of gout, hypertension and pityriasis rubra pilaris. At referral his adjusted total calcium concentration was 3.31mmol/L and he was mildly symptomatic. Renal impairment was present with eGFR 23 ml/min, and nephro-calcinosis was found on abdominal CT scans and renal ultrasound.

Initial results: 24hour urine calcium excretion was 26mmol/day, urine calcium creatinine ratio of 0.81, serum PTH 0.7pmol/L, 25 hydroxycholecalciferol 65nmol/L. Repeat total and ionised calcium after 3 weeks were 3.45mmol/L and 1.74mmol/L respectively. Intravenous saline, frusemide and pamidronate resulted in transient falls in total and ionised calcium. Further investigation revealed PTH-RP <1.0pmol/L, 1,25 dihydroxycholecalciferol 244pmol/L (reference range: 50-160pmol/L).

Non-contrast thoracic and abdominal CT scan showed perihilar lymph nodes of approximately 8mm, minimal abdominal lymphadenopathy and no splenomegaly. A highly unusual PET scan showed florid perihilar and subcarinal lymph node activity, multiple highly metabolically active areas most prominent in the proximal thighs, R latissimus dorsi, the sternoclavicular and sternomanubrial joints. Bone marrow, and flow cytometry of serum revealed no evidence of myeloma or lymphoma. Muscle biopsy confirmed granulomatous infiltration and high dose prednisolone therapy was commenced.

Discussion: Initially the severe hypercalcemia with hypercalciuria suggested malignancy rather than excessive 1,25 hydroxylation of vitamin D. Noteworthy features of this case for further discussion are (1) the severity of hypercalcemia accompanied by relatively minor perihilar lymphadenopathy consistent with early stage sarcoidosis, (2) the presence of granulomas within muscle which could have contributed to 1,25 hydroxylation of vitamin D and (3) the diagnostic utility of PET for investigation of hypercalcemia.

066

PRIMARY HYPERPARATHYROIDISM CAN BE DIFFICULT TO DIAGNOSE - TWO UNUSUAL CASES

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A male (23 yr) presented with a fractured femur and hypercalcaemia (3.6 mmol/L [RR 2.10 – 2.55]) after an episode of abdomen pain and vomiting. The serum PTH level was >263 pmol/L (RR 0.8-5.5 pmol/L), ALP 1352 U/L (RR 30-110 U/L), but paraprotein was undetectable. Skeletal survey showed multiple rib fractures. A Tc99m Sestamibi parathyroid scan revealed a bright spot in the right superior mediastinum; chest CT showed a 2x2 cm mass. Surgical mediastinal exploration, performed via a sternal split, because of the patient's short stature, retrieved benign thymic tissue, but found no parathyroid tumour. Surgery was subsequently performed via a cervical collar incision and a 4x5 cm mass, wrapped around the brachiocephalic vein in the posterior-lateral upper mediastinum, resected with difficulty. Serum calcium and PTH levels normalized post-surgery, while histology established a benign parathyroid adenoma. Markedly high PTH and ALP levels, associated with skeletal fractures are typically indicative of parathyroid carcinoma, therefore the present finding and location of an ectopic adenoma, and the difficulty in its removal, is rare.

A woman (84 yr) presented with severe hypercalcaemia (4.62 mmol/L [RR 2.10-2.55]) after admission for increasing lethargy and disorientation. The serum PTH level was 11.5 pmol/L (RR 0.8-5.5 pmol/L) and creatinine 235 μmol/L (RR 50-120). The patient received saline hydration and pamidronate (90 mg) infusion. Within 3 days serum calcium had decreased to 2.58 mmol/L. About 8 months later, serum calcium, PTH and creatinine levels were 2.44 mmol/L, 5.1 pmol/L and 144 μmol/L respectively. An initial Tc99m Sestamibi parathyroid scan revealed focal accumulation of tracer at the inferior right thyroid pole, suggestive of a parathyroid adenoma. Parathyroidectomy however, was deferred due to concerns about her age and co-existing CHD. The patient was also taking antacid preparations containing aluminium hydroxide and calcium carbonate prior to admission. Accordingly we believe that her initial severe hypercalcaemia resulted from milk-alkali syndrome overlying mild primary hyperparathyroidism.

067

BILATERAL ADRENAL INFARCTION DUE TO HEPARIN-INDUCED-THROMBOCYTOPAENIA-THROMBOSIS-SYNDROME (HITTS)

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We report two cases of *bilateral adrenal infarction* due to HITTS. The first case was also diagnosed with essential thrombocythaemia.

Mrs OS, a 74 year old woman was admitted with worsening right foot pain in association with gangrene of right 3rd and 5th toes on a background of osteoporosis, gastro-oesophageal reflux disease and long term smoking. Initial examination revealed regular pulse rate, normotensive, afebrile and good peripheral pulses. However, right 3rd and 5th toes looked ischaemic, purplish in colour, as well as some discoloration of right forefoot. Lower limb arterial dopplers showed no occlusion. IV heparin was commenced.

One week later, she had an episode of hypotension 80/40 mmHg, in association with "severe chest and upper abdominal pain radiated around to the back". This was subsequently diagnosed to be NSTEMI with troponin rise to 4.30 (<0.01). Following this, Mrs OS continued to have persistent lower abdominal pain and lower back pain, in association with persistent nausea and vomiting. Five days later, she was noted to be tachycardic, hypotensive, dyspnoeic, febrile and hypoglycaemic (BSL 2.7mmol/L), required ICU admission with intubation and ventilation.

Short synacthen test was abnormal with baseline cortisol 54 nmol/L peaking at 58 nmol/L; platelet count dropped from 978 to 31 in presence of anti-PF-4-heparin antibodies; bone marrow aspirate/trephine revealed diagnosis of essential thrombocythaemia. ACTH was elevated at 42.6 pmol/L (0.0-10.0). CT scan findings at onset of adrenal thrombosis in comparison to later in the illness will be shown.

In retrospect serum cortisol fell over the five days following the original hypotensive episode before adrenal insufficiency was diagnosed. The CT scan findings in a second case of adrenal infarction due to HITTS will be illustrated.

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068

A CASE OF REGIONAL OSTEOPOROSIS

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A 46 year old woman with a gradual onset of right foot pain, swelling and erythema is presented. There had been no prior history of trauma. Her pain worsened over a 2 month period to the point where she had difficulty weight bearing. Her right foot was swollen, warm and erythematous compared to the left and mobility was reduced at the ankle due to pain. There was no other joint or spinal involvement. Distal pulses in the lower limb were intact and there was no evidence of peripheral neuropathy. Biochemical investigations were essentially unremarkable with calcium, 25 OH VitD, iPTH and alkaline phosphatase within normal limits. Plain x-ray of the right foot and ankle revealed soft tissue swelling but no fractures or arthritic changes. A bone scan showed intense uptake at the right talus. MRI of the right ankle showed bone marrow oedema in the talar body and neck and a reactive joint effusion

in the distal tibia. These findings were consistent with regional osteoporosis. The patient was treated with alendronate 70mg/week and calcium and vitamin D replacement with considerable improvement in pain and function of her right foot over several months.

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ASEPTIC MENINGITIS ASSOCIATED WITH LYMPHOCYTIC HYPOPHYSITIS AND THYROIDITIS

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Lymphocytic hypophysitis (LH) is a rare inflammatory disorder characterized by lymphocytic infiltration throughout the pituitary gland. Previous cases have suggested that LH frequently has an autoimmune cause.

We reported a 37 year-old man presenting with diarrhoea, nausea, weight loss, low grade fever and headache who was initially diagnosed as having aseptic meningitis. Subsequently, by chance, he was found to have a homogenously enlarged pituitary gland on MRI and partial hypopituitarism with ACTH and gonadotrophin deficiency. Notably, his free thyroxine and tri-iodothyronine levels were elevated with a suppressed TSH and a suppressed thyroid technetium scan consistent with thyroiditis. Tissue autoantibodies including thyroid antibodies were negative. Enterovirus was initially isolated from faecal culture, pending further confirmation. Following introduction of hydrocortisone, he developed transient diabetes insipidus which spontaneously resolved after four months. Thyrotoxicosis resolved after five weeks and thyroxine was commenced as he developed secondary hypothyroidism. Testosterone replacement was commenced four months after diagnosis. Repeat MRI three months later showed a reduction in the size of pituitary gland which by six months had returned to normal size.

Based on clinical and radiological grounds, the diagnosis was consistent with lymphocytic hypophysitis with associated subacute thyroiditis. No evidence of associated autoimmunity was demonstrated. The initial non-specific symptoms associated with asceptic meningitis suggest a possible viral aetiology as a potential cause resulting in an inflammatory process involving both the pituitary and thyroid gland even though the original enteroviral cultures could not be confirmed.

070

AN INCIDENTAL FINDING - PHAEOCHROMOCYTOMA IN A PATIENT WITH VON RECKLINGHAUSEN'S DISEASE (NEUROFIBROMATOSIS TYPE 1)

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A 54 year old woman with Von Recklinghausen's disease was admitted to the coronary care unit with a non ST elevation myocardial infarct. Cardiac imaging showed a mass in the left ventricle. This was further investigated with CT which revealed bilateral adrenal masses.

MIBG imaging confirmed a 9cm right phaeochromocytoma. Due to concern regarding the nature and significance of the left adrenal mass, the patient underwent left adrenal vein sampling. This demonstrated a normal noradrenaline: adrenaline ratio. Due to ongoing suspicion of bilateral phaeochromocytomas, the patient underwent bilateral adrenalectomies. Histology confirmed bilateral phaeochromocytomas. A further incidental mass in the small bowel was also demonstrated on CT and resected. This was found to be a gastrointestinal stromal tumour.

Underlying genetic causes in patients presenting with apparently spontaneous pheochromocytomas or paragangliomas are common, being present in about one quarter.

Case discussion will include an overview of the four main genetic causes of phaeochromocytomas and paragangliomas, namely Neurofibromatosis Type 1, MEN Type 2, Von Hippel-Lindau disease, and Succinate Dehydrogenase Subunit mutations. The differing characteristics of phaeochromocytoma / paraganglioma in these conditions will also be discussed. The epigenetic phenomenon of imprinting and its relevance in SDHD will also be mentioned. The presentation will conclude with recommendations regarding which patients should be referred for genetic testing and the order in which these tests should occur.

071

CONTROL OF ANDROGEN RECEPTOR SIGNALING IN PROSTATE CANCER BY THE COCHAPERONE SMALL GLUTAMINE-RICH TETRATRICOPEPTIDE REPEAT CONTAINING PROTEIN ALPHA

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Although the androgen receptor (AR) is accepted as the major determinant of prostate cancer cell survival throughout disease progression, it is currently unclear how the receptor is able to sustain genomic signaling under conditions of systemic androgen ablation. Here we demonstrate that the evolutionarily conserved Hsp70/Hsp90 cochaperone, small glutamine-rich tetratricopeptide repeat containing protein alpha (αSGT), interacts with the hinge region of the human androgen receptor (AR) in yeast and mammalian cells. Manipulating cellular αSGT levels by overexpression and RNA interference revealed that this cochaperone acts to (i) promote cytoplasmic compartmentalization of the AR thereby silencing the receptors basal/ligand-independent transcriptional activity, (ii) regulate the sensitivity of receptor signaling by androgens, and (iii) severely limit the capacity of non-canonical ligands to induce AR agonist activity. Immunofluorescence, coactivator and chromatin immunoprecipitation analysis strongly suggest that these effects of αSGT on AR function are mediated by interaction in the cytoplasm and are distinct from the receptors response to classic coregulators. Quantitative immunohistochemical analysis of αSGT and AR levels in a cohort of 32 primary and 64 metastatic human prostate cancers revealed dysregulation in the level of both proteins during disease progression. The significantly higher AR:αSGT ratio identified in metastatic samples is consistent with the sensitization of prostate tumor cells to androgen signaling with disease progression, particularly in a low-hormone environment. These findings implicate αSGT as a molecular rheostat of *in vivo* signaling competence by the AR, and provide new insight into the determinants of androgen sensitivity during prostate cancer progression.

072

RAPID MOVEMENT OF PR INTO SUBNUCLEAR FOCI DEPENDS ON ASSOCIATION OF PR WITH CHROMATIN

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The progesterone receptor (PR) is a critical mediator of progesterone action in the female reproductive system. In addition to its pivotal role in normal physiology, exposure to exogenous progestins contributes to the development of breast cancer. Expressed in the human as two proteins, PRA and PRB, the receptor is a ligand-activated nuclear transcription factor. We have demonstrated that endogenous PR is present in discrete subnuclear foci in human breast and endometrium. Formation of foci is ligand-dependent in vivo and in vitro. Foci are associated with nascent RNA and are disrupted by transcriptional inhibitors, demonstrating their role in active transcription. This is supported by FRET analysis showing that PR foci contain the highest levels of PR dimers. PR foci are larger in endometrial cancers than in normal endometrium, and hormone-dependence is decreased. Chromatin remodelling using the HDAC inhibitor trichostatin A caused foci to become larger and to form independent of ligand. To determine whether the different chromatin structure in normal and cancer cells is a key determinant of PR foci, we used live cell imaging to compare foci formation in U-2OS osteosarcoma cells and in minimally transformed MCF-10A breast cells using fluorescently-tagged PRA and PRB. In both cell types, YFP-PRA and YFP-PRB were detected in prominent foci within 20 to 40 seconds of exposure to the progestin ORG2058. However, foci were larger in the transformed cells than in the cells with a more normal nuclear architecture. Mutation of the nuclear matrix targeting sequence resulted in prevention of PR binding to the matrix, and also prevented movement of PR into foci. Taken together, the demonstration of different sized foci in malignant and minimally transformed cells, and the reliance on nuclear matrix attachment for foci formation, suggest that chromatin structure is a critical component of normal PR transcriptional function.

073

IDENTIFICATION OF NOVEL PHARMACOLOGICAL ANTAGONISTS FOR LRH-1

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The orphan nuclear receptor Liver Receptor Homologue-1 (LRH-1) has been implicated in breast cancer progression due to its ability to stimulate aromatase expression in breast adipose tissue. LRH-1 modulates tissue-specific estrogen production in the adipose stromal cells via transcriptional activation of promoter II (PII) of the aromatase (*CYP19*) gene. LRH-1 also has direct effects on cancer cell proliferation *via* direct stimulation of expression of G1 cyclins, with consequent induction of cell cycle progression. As such, LRH-1 is an attractive target for drug development; the aim of this project to identify antagonists LRH-1. An *in silico* screen of

approximately 4 million small drug-like compounds was conducted to identify candidates predicted to bind and potentially inhibit LRH-1 activity. 100 compounds predicted to bind the cofactor-binding- and ligand-binding domains with high specificity were selected for screening *in vitro*. The direct interaction of test compounds with LRH-1 was tested; COS7 cells were transfected with a GAL4-LRH-1 fusion construct and the yeast-specific luciferase reporter containing GAL4-response elements. A dose-dependent inhibition (0.01, 0.1, 1 and 10 μM) of LRH-1 activity was demonstrated with 15 compounds. Treatment with 1 and 10 μM concentrations demonstrated 50-95% inhibition in these potential LRH-1 antagonists. To validate this observation, full-length LRH-1 was cotransfected with the *CYP19* PII luciferase reporter construct. Three compounds were found to suppress *CYP19* PII transcriptional activity in a dose dependent manner, achieving inhibition (50-90%) at 10 μM. Specificity of compounds was controlled for by the inclusion of compounds that did not inhibit LRH-1 activity; these were found to not suppress *CYP19* PII transcriptional activity. The specificity of these compounds to inhibit LRH-1 over related nuclear receptors is currently under investigation. Identification of a novel class of LRH-1 inhibitors will improve understanding of the role of LRH-1 in breast tumorigenesis. Ultimately, these antagonists could allow the development of new breast cancer therapeutics.

074

SKELETAL MUSCLE AS A TARGET FOR REGULATION OF FAT MASS AND METABOLISM IN MALE ANDROGEN RECEPTOR KNOCKOUT MICE

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We are using our androgen receptor knockout (ARKO) mouse model, which lacks the second zinc finger of the DNA binding domain, to study the mechanisms through which androgens regulate fat mass and metabolism. ARKO males have decreased body mass but increased fat mass compared to wildtype males (subcutaneous fat increased by 75.3% ($p<0.001$) and renal fat increased by 35.6% ($p<0.05$) in 12 week ARKO males). This demonstrates that AR-dependent mechanisms regulate adiposity in males. At 9 and 12 weeks of age there is no difference in fed levels of serum glucose (currently measuring fasting levels). Two potential targets of androgen action are adipose tissue and skeletal muscle. To identify androgen-responsive genes in muscle, microarray analysis was performed on RNA from gastrocnemius muscle in 9 week ARKO and wildtype males (n=2/group). Four metabolic genes showing >1.5 fold increased change in expression in ARKO males were examined by quantitative real-time PCR to confirm these observations, using RNA from the gastrocnemius of 12 week ARKO and wildtype male mice (n=11-12/group). Of the four genes examined, only one gene had increased expression in ARKO males (158.5% increase ($p<0.001$)), consistent with microarray data. Further real-time PCR analysis will be performed on other metabolic genes showing altered expression on microarray analysis in ARKO males. Preliminary data from 12 week muscle-specific ARKO males (n=8/group) shows no significant change in fat mass compared with floxed AR controls, although further analysis is required to confirm this finding. These data suggest that adipose tissue may be the major target for androgen action in fat metabolism, which will be examined using adipose tissue specific ARKO mice.

075

GLUCOCORTICOID RECEPTORA, PCREB-1 AND CBP BINDING ON THE PROSTAGLANDIN ENDOPEROXIDE H SYNTHASE (PGHS-2) PROMOTER OF TERM AMNION *IN VIVO*.

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Expression of PGHS-2 (PTGS2), the rate limiting prostaglandin biosynthetic enzyme, increases in the human amnion in late gestation, leading to the increased production of prostaglandins that stimulate labour. PGHS-2 expression is controlled by gene activity via mechanisms involving epigenetic modifications of chromatin and regulatory transcription factor binding to the promoter. We have shown previously that histone acetylation is increased at the proximal 1000bp region of the PGHS-2 promoter in term amnion indicating that chromatin structure is open near the transcription initiation site before and after labour. In this study, we have determined the binding of several regulatory transcription factors to this region *in vivo* to assess their involvement in PGHS-2 transcription control. Amnion tissues were collected after spontaneous labour and elective Caesarean section at term, and chromatin immunoprecipitation was performed with fresh tissues using antibodies against glucocorticoid receptor-alpha (GRα), phosphorylated cAMP-response element binding protein (pCREB-1) and CREB-binding protein (CBP, a histone acetyl transferase). The immunoprecipitated DNA was analysed by real-time PCR using eight primer pairs covering the first 2500bp region of the PGHS-2 promoter. We found GRα binding to the proximal 1000bp region ($p<0.05$, ANOVA), where chromatin structure is permissive, but no consensus GRα -response sequence (GRE) is present. No GRα binding was detected in the upstream restrictive promoter region. GRα binding was most pronounced at 213-222 bases upstream of the transcription initiation site. This site bound pCREB-1 and CBP as well. Further, GRα and pCREB-1 binding, but not CBP binding, diminished after labour. The data suggest that glucocorticoids and pCREB-1 are involved in the up-regulation of PGHS-2 gene before term labour. CBP may act as a transcriptional coactivator and histone acetyl transferase maintaining PGHS-2 gene activity and permissive chromatin structure before and during labour. The presence of a non-canonical GRE in the PGHS-2 promoter remains to be explored.

076

RORALPHA, AN ORPHAN NR MODULATOR OF METABOLISM IN THE LIVER AND PERIPHERAL METABOLIC TISSUES.

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Impaired RORalpha expression and function in staggerer mice (sg/sg) results in a dyslipidemic phenotype. These mice display decreased adiposity, serum cholesterol (total and HDL), triglycerides and free fatty acids. Our initial studies indicate that RORalpha4 is the predominant isoform in wild-type mice, and that expression of RORalpha (1 and 4) transcripts are attenuated in staggerer mice. We utilized this mouse model to investigate the role of RORalpha in the metabolic adaptation to changes in dietary status in the liver and peripheral metabolic tissues. In this context we observed that dysfunctional RORalpha expression impaired the nutrient dependent regulation of genes associated with anabolic and catabolic lipid homeostasis in the liver, skeletal muscle and white adipose tissue.

077

CHARACTERISATION OF MYOBLAST- AND MYOFIBRE-SPECIFIC ANDROGEN RECEPTOR KNOCKOUT MICE TO IDENTIFY MECHANISMS OF ANABOLIC ACTIONS OF ANDROGENS IN SKELETAL MUSCLE

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To identify the direct anabolic actions of androgens on skeletal muscle, we have generated two lines of muscle-specific androgen receptor (AR) knockout (mARKO) mice. mARKO mice have an in-frame deletion in the DNA binding domain, and in the α-actin-cre mARKO line the AR is deleted in both proliferating myoblasts and post-mitotic myofibres, but in the MCK-cre mARKO line the AR is deleted only in post-mitotic myofibres. In both mARKO lines, quantitative real-time PCR shows that AR gene expression is reduced by ~98% in skeletal muscle compared with wildtype controls.

Skeletal muscle mass is reduced in 12 week α-actin mARKO and MCK mARKOs, compared with wildtype, AR^{lox} hemizygous and cre heterozygous male littermate controls (one-way ANOVA). The highly androgen-dependent perineal muscle, the levator ani (LA), is reduced in mass by approximately 50% in both lines ($p<0.001$). The mass of hindlimb muscles shows a smaller magnitude reduction in both mARKO lines, including the fast-twitch tibialis anterior (α-actin mARKO 13.9%↓, $p<0.001$; MCK mARKO 14.9%↓, $p<0.001$), and slow-twitch soleus (α-actin mARKO 8.5%↓, $p<0.05$; MCK mARKO 11.9%↓, $p<0.05$). Using real-time PCR, we have quantitated the expression of the polyamine biosynthetic genes, S-adenosylmethionine decarboxylase (Amd1) and ornithine decarboxylase (Odc1), which are down-regulated in muscle from our global ARKO mice. Expression of both genes is also significantly reduced in the MCK mARKO gastrocnemius (GAST) and LA muscles versus wildtype (GAST: Amd1 5-fold↓, $p<0.001$; Odc1 3-fold↓ $p<0.01$; LA: Amd1 15-fold↓ $p<0.001$).

These data confirm our hypothesis, that androgens have direct anabolic actions through AR-dependent gene regulation in skeletal muscle. The similarity of muscle phenotype in both mARKO mouse lines suggests that myoblasts are not a major target for the anabolic actions of androgens. Our results suggest that regulation of polyamine biosynthesis in skeletal muscle is a major pathway of the anabolic actions of androgens in skeletal muscle.

078

DISRUPTED GLUCOCORTICOID AND CAMP SIGNALING VIA CREB CAUSE DISTINCT BUT OVERLAPPING PHENOTYPES IN THE DEVELOPING MAMMALIAN LUNG

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The mammalian lung develops via integrated preprogrammed cell differentiation and responses to systemic hormones. The glucocorticoid cortisol provides important developmental cues for the late maturation of the human fetal lung and antenatal synthetic glucocorticoids are used to treat respiratory distress syndrome suffered by preterm babies. The intracellular cAMP signaling pathway is important for promoting synthesis of lung surfactant yet little is known of its wider role in the differentiation and development of the respiratory system. To dissect the specific role of cortisol and cAMP signaling in fetal lung development, we have analyzed mice with targeted null mutations for either the glucocorticoid receptor (GR) or cAMP responsive element binding (CREB) protein gene (1, 2). In the absence of functional GR, lung development is severely retarded; the lungs are hypercellular with reduced septal thinning. Histological and morphometric analysis reveal dramatically reduced proportions of differentiated type-1 AECs. CREB null mice do not survive birth and have a more pronounced defect in the developing lung. The lung is very condensed with marked hypercellularity and hyper-proliferation. There is no detection of differentiated type-1 AECs and ablated expression of upper-airway respiratory markers. Whole genome expression microarray analysis has revealed both glucocorticoid and CREB-regulated gene targets that begin to map gene networks important for respiratory development and function. These results demonstrate that cortisol and cAMP signaling pathways are essential for the embryonic development of the lung and have distinct yet overlapping roles in respiratory epithelial cell differentiation.

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079

PROSPECTIVE EVALUATION OF A PROTOCOL FOR REDUCED GLUCOCORTICOID REPLACEMENT IN TRANSSPHENOIDAL PITUITARY ADENOMECTOMY FOR NON-CUSHING'S TUMOURS: PROPHYLACTIC GLUCOCORTICOID REPLACEMENT IS UNNECESSARY IN LOW-RISK CASES.

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Most pituitary surgery centres prescribe perioperative glucocorticoids to subjects undergoing transsphenoidal pituitary adenomectomy (TSA) despite evidence-based guidelines recommending against this practice (1). We have implemented a protocol based on these guidelines at The Royal Melbourne Hospital and report outcomes for 56 consecutive TSAs performed between March 2004 and April 2006. Glucocorticoid was withheld in 44 'low-risk' cases characterised by no evidence of preoperative glucocorticoid deficiency and/or pituitary apoplexy. Using daily clinical examination and a morning serum cortisol threshold of 250nmol/L to guide postoperative glucocorticoid requirement, we identified and treated two glucocorticoid-dependent subjects before they developed significant complications. None of the remaining 42 'low-risk' cases required glucocorticoid on discharge from hospital. There were no incidents of clinical adrenal insufficiency in these patients during a follow-up of at least twelve months. We assessed adrenal function in 35 of these patients between 12 and 24 months after surgery. All had normal short Synacthen tests. Glucocorticoid use during inpatient stay and one month after surgery was significantly reduced when compared with a cohort of 47 consecutive TSAs performed before implementation of our protocol. We conclude that our protocol is not only safe but also simplifies assessment of the hypothalamo-pituitary-adrenal axis following TSA, with consequent reductions in unnecessary and potentially harmful glucocorticoid treatment.

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080

VASOPRESSIN-SENSITIVE ACTH-INDEPENDENT MACRONODULAR ADRENAL HYPERPLASIA (AIMAH): A RARE CAUSE OF CUSHING'S SYNDROME. CLINICAL AND GENETIC STUDIES OF A LARGE SOUTH AUSTRALIAN KINDRED.

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Introduction: Vasopressin-sensitive AIMAH (VPs-AIMAH) is a rare form of adrenal Cushing's. Affecteds demonstrate a non-ACTH-dependent rise in serum cortisol following VP stimulation. Familial forms are described, though the genetics are not defined. We have a 37-member kindred with VPs-AIMAH – 3 siblings presented with either Cushing's or adrenal tumours and subclinical Cushing's, suggesting autosomal dominant inheritance.

Hypothesis: (1) VPs-AIMAH is an autosomal dominant disorder; (2) HPA axis abnormalities or structural adrenal changes precede clinical Cushing's. AIMS: To define early stages of the clinical and biochemical phenotype of VPs-AIMAH to enable early detection of affecteds, and to determine its genetic cause. This may improve understanding of the mechanisms of adrenal tumorigenesis.

Mehtods: Kindred members underwent annual assessment for Cushing's or adrenal tumours. Genomic studies include: sequencing the VP receptor gene; linkage studies and candidate gene analysis. A genome-wide search may ultimately be necessary. Adrenal tumour studies include VP receptor expression studies, and comparative genomic hybridisation for chromosomal aberrations. Studying other affected kindreds may facilitate detection of the causative mutation.

Results: Sequencing the VP receptor gene in 2 affecteds has not identified a germline mutation. No VP receptor gene mutation was identified in tumour DNA from the proband. One affected is eucortisolaemic after unilateral adrenalectomy. A cortisol day curve postoperatively demonstrated a preserved, but blunted, circadian rhythm, despite persistent ACTH suppression. Preoperative investigations suggested bilateral adrenal hyperfunction. Another individual is not clinically Cushingoid, but has HPA axis abnormalities biochemically and structural adrenal changes on imaging; two others have had abnormal responses to vasopressin. In total, 3 members are affected, 5 are categorized as possibly affected and one is unaffected.

Conclusions: VP-s AIMAH may be familial and in our kindred is likely to be autosomal dominant. Our data suggest that mild non-Cushingoid preclinical states of VPs-AIMAH can be detected using endocrine and imaging studies. This should facilitate the eventual discovery of the heritable abnormality causing AIMAH and may improve understanding of adrenal tumorigenesis.

081

EFFECT OF CORTICOTROPIN-RELEASING HORMONE (CRH) AND CORTISOL ON DESENSITIZATION OF THE ADRENOCORTICOTROPIN (ACTH) RESPONSE TO ARGININE VASOPRESSIN (AVP) IN OVINE ANTERIOR PITUITARY CELLS

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CRH and AVP are major physiological stimulators of ACTH secretion from the anterior pituitary, while glucocorticoids act as inhibitors. In addition to acting alone, CRH, AVP and glucocorticoids interact with each other to regulate ACTH release in response to stress. Prolonged or repeated stimulation results in attenuated ACTH responsiveness, or desensitization. The aim of this study was to investigate the effects of interactions between CRH, AVP and cortisol on desensitization of the ACTH response to AVP.

Perifused ovine anterior pituitary cells were stimulated with three 5-min pulses of 100 nM AVP at 120, 200 and 280 min, and desensitization was induced by a 15 min pre-treatment with AVP immediately before the second AVP pulse. In the absence of either CRH or cortisol, pre-treatment with 5 nM AVP reduced the response to the second AVP pulse (to 66.7±1.9% of control, n=10, $P<0.0001$), but pre-treatment with 0.5 nM AVP had no effect. When cells were continuously exposed to CRH (0.2 nM) from 80 min, 0.5 nM AVP pre-treatment reduced the response to the second pulse (to 66.7±2.2% of control, n=6, $P<0.0001$). Continuous perifusion with cortisol (100 nM) from 0 min caused a significantly smaller reduction in the response to the second AVP pulse following 5 nM AVP pre-treatment compared with that seen in its absence (78.4±1.7% cf. 66.7±1.9% of control; n=10, $P<0.001$). In contrast, continuous exposure to both CRH and cortisol resulted in a greater reduction in the response to the second AVP pulse following 5 nM AVP pre-treatment compared with that obtained in the absence of these two hormones (46.5±1.7% cf. 66.2±1.7 of control; n=8, $P<0.0001$).

These data indicate that desensitization of the ACTH response to AVP can be modulated by CRH and/or cortisol: desensitization is reduced by cortisol, but amplified by either CRH alone, or CRH and cortisol together.

082

SKELETAL MUSCLE 11B HYDROXYSTEROID DEHYDROGENASE TYPE 1 IS UPREGULATED FOLLOWING ELECTIVE ABDOMINAL SURGERY

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Surgical trauma induces insulin resistance in normal and glucose intolerant individuals, which lasts post-operatively for approximately one week. Skeletal muscle is thought to be the main site of insulin resistance, and abnormalities of the insulin signalling cascade have been demonstrated in the acute post-operative period. The aetiology of this intracellular insulin resistance is unknown. Cortisol, which has traditionally been implicated in the causation of peri-operative insulin resistance returns to normal within 72 hours of surgery. We hypothesized that the intracellular effect of cortisol might be enhanced and prolonged by upregulation of 11β hydroxysteroid dehydrogenase type 1 (11β HSD1) which converts inactive cortisone to cortisol. We therefore investigated the time course of skeletal muscle 11β HSD1 enzyme activity and mRNA expression relative to plasma cortisol levels following abdominal surgery. Eight subjects without a history of diabetes underwent frequent plasma hormone sampling, and muscle biopsy of vastus lateralis at baseline and on day 5 following elective laparoscopic cholecystectomy. Measurements included 11β HSD1 and H6PDH mRNA levels by quantitative RT-PCR and enzyme activity by % conversion of ^{3}H cortisone or cortisol respectively and plasma glucose, insulin, free fatty acids (FFA), TNFα and cortisol. β-cell function in the fasting basal steady state was calculated as 20 x [fasting insulin (m U/mL)/ fasting glucose (mmol/L)] – 3.5. 11β HSD1 activity was significantly increased on day 5 after surgery (14.7 ± 2.1% vs 20.4 ± 3.2%, P=0.005). In contrast, 11β HSD1 mRNA levels did not change significantly. Serum cortisol (P=0.027), FFA (P=0.01) and glucose (P=0.004) rose rapidly following surgery and returned to baseline values by 24 hours post surgery. The b -cell function index fell significantly, with the nadir on the first post-operative day (P=0.014). This is the first study to demonstrate an upregulation of skeletal muscle 11β HSD1 activity in response to a physiological stress. Sustained activation of this enzyme may increase tissue levels of cortisol and contribute to post-operative skeletal muscle insulin resistance.

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FACTORS DETERMINING INADEQUATE HYPOGLYCAEMIA DURING INSULIN TOLERANCE TESTING AFTER PITUITARY SURGERY

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Introduction: Insulin tolerance test (ITT) is the "gold standard" test for assessing pituitary/adrenal status after pituitary surgery. Some patients fail to achieve adequate hypoglycaemia following a standard dose of intravenous insulin. Among patients with acromegaly and Cushing's disease (CD), persistence of disease may contribute to inadequate insulin-induced hypoglycaemia. Aims: To identify factors that predispose to failure to achieve adequate hypoglycaemia after pituitary surgery during an ITT. Methods: We reviewed ITTs performed after pituitary surgery from 1998-2007. A review was undertaken to identify patients in whom adequate hypoglycaemia (blood glucose ?2.2 mmol/L) was not achieved (insulin-insensitive group; n=32, 14F) following a standard dose of insulin (0.1 Units/kg). An equal number of gender-matched patients, who achieved adequate hypoglycaemia (insulin-sensitive group) and required glucose rescue with oral and/or intravenous glucose were selected as controls. Analyses were performed to determine if

weight, baseline glucose or underlying diagnosis (acromegaly or CD) influence the ability to achieve hypoglycaemia during an ITT. Data are reported as mean±SD. Results: In the whole group, insulin-insensitive patients had significantly higher body weight (89.3±19.3 kg vs 76.9±19.7, p<0.01), fasting blood glucose (5.4±0.7 mmol/L vs 4.4±0.4, p<0.0001), and peak cortisol levels (554±292nmol/L vs 397±196, P=0.01). There was no difference between the groups in peak growth hormone response (11.2±10.8mU/L vs 11.9±17.6, p=NS). In patients with acromegaly or CD, fasting blood glucose and peak cortisol levels were not significantly different between the insulin sensitive and insensitive subgroups. However, the insulin insensitive subgroup had a higher proportion of patients with persistent disease (83% vs 33%; p<0.05). Conclusion: Patients with higher body weight, fasting blood glucose and peak cortisol response, and persistent acromegaly or CD require a higher insulin dose to achieve hypoglycaemia. Adequate response to one dose of insulin may be indicative of the likelihood of cure of acromegaly or CD following pituitary surgery.

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INHIBIN A BLOCKS GROWTH/DIFFERENTIATION FACTOR (GDF)-9 ACTION IN ADRENOCORTICAL CANCER (AC) CELLS

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GDF-9 signals via BMP type II receptor (BMPRII) in combination with the TGF- b type I receptor, ALK5 (*1*). Although known as an oocyte factor, GDF-9 is expressed in other tissues, including adrenocortical cancer cells (*2*), the pituitary and testis (*3*). We have tested the hypotheses that i) GDF-9 has actions on inhibin target cells (adrenocortical cells, pituitary gonadotrophs and testicular somatic cells), and ii) inhibin A and/or B oppose the actions of this BMPRII-selective ligand.

Cultures of mouse AC, Leydig-like TM3, Sertoli-like TM4 and LβT2 gonadotroph cells all expressed mRNA encoding GDF-9, BMPRII and ALK5. In AC cells, GDF-9 (50 ng/ml) suppressed insulin-stimulated *Cyp17* mRNA expression to 21±4% of control (mean±SEM, *n*=3) with an IC50 of 2-5 ng/ml (*n*=2). This inhibition was partly blocked by 1 nM inhibin A (59±5% of control) but not 1 nM inhibin B (21±2% of control). GDF-9 and activin increased luciferase expression in pGRAS-transfected AC cells (1.9- and 2.4-fold, respectively, averages of *n*=2), and the actions of both were blocked by inhibin A, whereas inhibin B only blocked activin. GDF-9 lacked such actions in similarly transfected TM3 and TM4 cells. SB431542, an ALK4/5/7 inhibitor, blocked the actions of activin A and GDF-9, but not BMP-6 (2 nM), in AC cells. GDF-9 treatment for 3 d neither increased FSH production and secretion nor blocked activin stimulation of these FSH levels in primary rat anterior pituitary cell cultures.

In summary, GDF-9 stimulates BMPRII/ALK5-expressing AC cells during 24 h, and its action can be selectively blocked by inhibin A, but it has little effect on pituitary FSH levels. We conclude that suppression of adrenocortical *Cyp17* expression by locally produced GDF-9 may be subject to antagonism by inhibin A, but not inhibin B. The pituitary target for locally produced GDF-9 is unlikely to be the gonadotroph.

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085

ARTERIO-VENOUS TRANS-ORGAN SAMPLING IN NORMAL AND HEART FAILURE SHEEP: DETERMINING THE SOURCE OF CIRCULATING HORMONES.

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One way of delineating a potential role for a hormone in disease pathophysiology is to investigate circulating levels of the peptide in normal and diseased states. Although plasma levels of a number of novel hormones such as urocortin 1 (Ucn1) and urotensin II (UII) are reported to be elevated in heart failure, the source of these rises is yet to be determined. In addition there have been no reports of regional changes in plasma C-type natriuretic peptide (CNP) and amino terminal CNP (NT-CNP). The plasma concentrations of a peptide are the net result of endogenous secretion/production and clearance. We therefore performed trans-organ arterio-venous (A-V) sampling in anaesthetised sheep before and after induction of pacing-induced experimental heart failure. We have then employed sensitive, validated radioimmunoassays to measure plasma natriuretic peptides (ANP, BNP, CNP & NT-CNP), Ucn1 and UII. Results show that ANP and BNP plasma concentrations are sourced from a single organ (the heart) and are subject to substantial extraction across most tissue beds. In contrast, multiple tissues including liver, heart, hind limb and kidney contribute to circulating CNP. Given that A-V gradients for NT-CNP were similar, this is likely to represent de novo secretion. Circulating levels of CNP and NT-CNP were raised in heart failure but to much lesser degree than ANP and BNP. Arterial plasma level of Ucn1 measured 15.2+0.5 pmol/L in normal sheep and increased significantly in heart failure to 19.1+1.6 (p<0.05). Small but significant positive A-V gradients were observed across the hepatic and renal tissues in both states. Plasma UII levels measured in the low picomolar range in normal sheep and significant A-V gradients were observed across the heart (36%), liver (40%) and kidney (44%). These studies elucidate the circulating source of circulating CNP, NT-CNP, Ucn1 and UII. In conclusion, A-V regional sampling combined with sensitive assays provides information on the source of circulating hormones.

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CORTISOL EFFECTS ON FOOD INTAKE, LEPTIN AND ADIPOSITY ARE DEPENDENT ON SEASON IN THE EWE

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Glucocorticoids play a protective role on homeostatic systems, but chronic high levels have detrimental effects on health, including severe weight loss or weight gain and impaired fertility. We investigated the effect of chronic elevation of cortisol on food intake, adiposity and endocrine parameters in ovariectomised ewes. Since photoperiod impacts on food intake and adiposity, we investigated the effect of cortisol during periods where food intake and bodyweight were at a zenith (January: JAN) or nadir (August: AUG). Animals were treated (i.m.) with either ACTH (0.5 mg Synacthen Depot) or saline daily for 4 weeks (n=5-6/group). Blood samples were taken before and after treatment to measure plasma levels of cortisol, luteinising hormone (LH), follicle stimulating hormone (FSH) and leptin. Truncal adiposity was determined using dual-energy X-ray absorptiometry. ACTH increased levels of cortisol in JAN ($P<0.01$) and AUG ($P<0.005$). ACTH increased ($P<0.05$) the LH inter-pulse interval in JAN and AUG, and reduced ($P<0.05$) LH pulse amplitude in JAN only; there was no effect of ACTH on the mean plasma levels of LH or FSH in JAN or AUG. Thus, there was little effect of photoperiod on the responsiveness of gonadotropins to ACTH. During JAN, ACTH transiently reduced ($P<0.05$) food intake, whereas ACTH increased ($P<0.05$) food intake during AUG. Leptin levels were lower ($P<0.05$) in AUG than in JAN, and ACTH increased ($P<0.05$) leptin levels in AUG only. There was no effect of ACTH on adiposity in JAN or AUG. In spite of this, changes in truncal adiposity were correlated to ACTH-induced cortisol secretion (as determined by area under the curve) in JAN ($R^2= 0.81$, $P<0.05$) and AUG ($R^2= -0.78$, $P=0.06$); photoperiod appeared to determine the direction of adipose gain or loss. We conclude that, in sheep, effects of chronic cortisol elevation on food intake, adiposity and leptin levels are dependent on photoperiod.

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RECIPROCAL REGULATION OF BONE METABOLISMS

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That gonadal failure triggers osteoporosis while obesity protects from it, this led us to hypothesize that there was a common endocrine control of bone mass, body weight and reproduction. In the first phase of this work we aimed at demonstrating that there was a control of bone mass by hormones regulating body weight and reproduction. We showed that the adipocyte-derived hormone leptin regulates bone mass following its binding to hypothalamic neurons and through the use of two distinct neural mediators, the sympathetic tone and CART (cocaine amphetamine regulated transcript). Bothe of these mediators act on osteoblast whose proliferation and function they regulate through distinct molecular pathways. More recently we asked the reverse question namely are osteoblast regulating energy metabolism? In other words in bone an endocrine organ? We will present at the meeting experimental evidence indicating that osteoblasts do regulate energy metabolism albeit in ways that we did not anticipate.

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THE EFFECT OF TESTOSTERONE & SEASON ON PREPROOREXIN MRNA EXPRESSION IN THE HYPOTHALAMUS OF THE RAM

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The neuropeptide, orexin, may provide a link between nutrition and reproduction, since it stimulates food intake (1) and also synapses with GnRH neurons (2). How orexin is regulated is poorly understood, however. In a previous study (3), we showed that both testosterone and season influence mRNA expression of another neuropeptide, enkephalin, in the preoptic area and hypothalamus of rams. Using tissue from the same study, we tested the hypothesis that testosterone and/or season modulate preproorexin mRNA expression in specific areas of the hypothalamus in the ram. Adult Romney Marsh rams were castrated either during the 'breeding' season or 'non-breeding" season and 1 week later received intramuscular injections of either peanut oil (vehicle) or testosterone propionate (8mg/12h for 7 days) (5/group). Blood samples taken every 10min for 12h were assayed for plasma LH and testosterone. PreProOrexin mRNA expression was quantified in hypothalamic sections by *in situ* hybridisation using a ^{35}S-labelled riboprobe and computer-aided image analysis. Plasma testosterone levels were higher in testosterone propionate-treated than oil-treated sheep. Mean plasma LH concentrations were reduced and the interpulse interval for LH pulses was greater in testosterone propionate-treated wethers compared to oil-treated wethers, with no change in LH pulse amplitude. PreProOrexin mRNA-containing cells were found in the perifornical area, lateral hypothalamus, zona incerta and ventromedial hypothalamus. There was no difference in the number of labelled cells/mm^2 between any of the treatment groups in any of regions tested. Preliminary evidence suggests that testosterone altered the number of silver grains per cell in the perifornical area. The data from the cell numbers suggest that neither season nor testosterone exert a significant effect on the regulation of preproorexin mRNA levels in the hypothalamus of the male sheep.

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(3) Scott et al. Biol Reprod 69:2015-2021 2003

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EFFECT OF CONCENTRATE FEEDING ON INSULIN RESPONSE POSTPARTUM IN GRAZING DAIRY COWS

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The effects of concentrate feeding on the insulin response to a infusion of intravenous glucose was determined in dairy cows. Sixty, mixed-aged, Holstein-Friesian cows were fed pasture and randomly allocated at calving to 0, 3, or 6 kg dry matter/day of pelleted concentrate. Five weeks after calving, all animals received an infusion of 300 mg glucose/kg live weight by intravenous catheter. Blood samples were collected at -2, 0, 2, 4, 6, 8, 10, 12, 15, 18, 20, 23, 26, 30, 35, 40, 50, 60, 90, 120, 150, 180, 210 and 240 min, relative to the time (T) of the infusion, for subsequent measurement of glucose and insulin concentrations. Glucose tolerance was measured by calculating glucose fractional turnover rate, half-life and area under the curve (AUC) 60 and 120 min after infusion. Insulin response to the glucose load was determined by insulin peak concentration, insulin concentration increment and AUC 60 and 120 min after infusion. The relationship of the glucose and insulin parameters with the post-partum anovulatory interval (PPAI) was also determined.

Glucose turnover rate was greater ($P<0.003$) on the 6 kg diet compared with the other diets, whereas glucose half life was shorter ($P<0.021$). AUC was lowest for the 6 kg concentrate diet at T60 ($P<0.01$) and T120 ($P<0.012$) compared with the 0 or 3 kg diets. There was no difference ($P>0.05$) in insulin response to the glucose load caused by the three diets as measured by insulin peak concentration, insulin concentration increment and AUC at T60 or T120. Correlations of these parameters with PPAI were not statistically significant.

While glucose tolerance was altered by feeding the 6 kg dry matter concentrate diet, this did not alter the insulin response to the glucose load in any of the three treatment groups. Therefore concentrate feeding did not appear to alter the insulin response of grazing dairy cows during early lactation.

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EFFECTS OF HIGH DIETARY PHYTOESTROGEN INTAKE ON OVARIAN INCLUSION CYST FORMATION IN INCESSANTLY OVULATED CD-1 MICE

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The presence of fluid-filled "inclusion" cysts in the ovary is a known risk factor for epithelial ovarian carcinogenesis. Cysts are thought to arise after entrapment of surface epithelium in the cortex during ovulation. However cysts have been observed in CD-1 mouse ovaries subjected to either repeated pregnancy or incessant ovulation for >6 months, irrespective of total ovulation number and most appear to be dilated rete ovarii tubules at the ovarian hilus [1] . This experiment aimed to determine the contribution of dietary phytoestrogen to inclusion cyst formation in CD-1 mice.

Incessant ovulation was induced from weaning until 8-months of age by housing CD-1 mice in screened cages [1] . Mice were fed a synthetic diet containing approximately 100 mg genistein and 70 mg daidzein/kg chow, derived from soy products (S; n=10). Controls were fed a nutrient-matched diet containing no phytoestrogens (C; n=9). On dissection, one ovary per mouse was fixed in 4% paraformaldehyde and serially sectioned at 4 µm. One section every 100 µm was stained with haematoxylin and eosin and examined for cysts and invaginations. Inclusion cysts were observed in 9/10 ovaries from S mice and in 4/9 control ovaries ($p<0.05$, χ 2 : average cyst number/ovary, C group = 0.50 ± 0.53 s.d.; S group = 2.2 ± 2.4; $p<0.05$, 2-tailed Mann-Whitney U test). All C group cysts originated at the ovarian hilus, whereas 7/22 of S group cysts were cortical. Estimated cyst volume and surface invagination number did not differ between groups.

We conclude the development of both hilar and cortical ovarian inclusion cysts, but not cyst size or surface invagination rate, is stimulated by dietary phytoestrogen intake in incessantly ovulated CD-1 mice.

(1) Fleming JS, et al. Reprod Biol Endocrinol 2007; 5: 14

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STRESS-LIKE LEVELS OF CORTISOL DO NOT AFFECT THE ONSET OR DURATION OF OESTRUS IN EWES

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Cortisol can impair the neuroendocrine regulation of the oestrous cycle in ewes by delaying or abolishing the LH surge. Nevertheless, it is unknown whether cortisol can disrupt the display of sexual behavior (oestrus) in ewes during the oestrous cycle. We tested the hypothesis that cortisol will delay the onset and/or reduce the duration of oestrus in ewes. Ovariectomised ewes (n=12) were induced into oestrus. All ewes were administered 20mg of progesterone via intramuscular (*i.m.*) injection every second day for 12 days and 48h following the final progesterone injection an *i.m.* injection of oestradiol benzoate (25µg) was administered. Catheters were inserted into both jugular veins. One catheter was used for a 30h intravenous infusion of cortisol (250µg/kg/hr) (n=6)

or vehicle (saline) (n=6) and the remaining catheter was used for blood collection. Infusion with cortisol or vehicle began at the time of the oestradiol benzoate injection and blood samples (5ml) were collected every 6h to examine plasma concentrations of cortisol. Following the injection of oestradiol benzoate, the sexual behaviour of each ewe was recorded in a 5 minute test with a ram every 6h. Ewes were moved into a pen where a ram was located and specific sexual behaviors of the ewe and ram were recorded. The mean (+SEM) plasma concentrations of cortisol were measured using radioimmunoassay and the mean (+SEM) time of onset and the duration of oestrus were calculated and examined for all ewes. Mean (+SEM) plasma concentrations of cortisol were significantly higher in ewes treated with cortisol (120+20.1ng/ml) compared with control ewes (16+1.0ng/ml). There was no significant difference in the mean (+SEM) onset of oestrus for ewes treated with vehicle (18+2.4h) or cortisol (19.8+2.2h), likewise for the duration of oestrus, there was no significant difference between control (32.4+2.4h) and cortisol treated ewes (29+3.6h). In contrast with the effects of cortisol on LH secretion, these results suggest that cortisol does not impair the onset or duration of oestrus in ewes.

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EFFECTS OF LETROZOLE ON THE PHENOTYPE OF ADULT AROM+ FEMALE MICE

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The aromatase overexpressing (AROM+) mouse is a model with an imbalance in sex hormone metabolism that was generated by expressing human aromatase under the human ubiquitin C promoter. The female AROM+ mice are fertile. The aim of this study was to determine the effect of administration of letrozole (non-steroidal aromatase inhibitor; Novartis International Pharmaceutical Ltd., Ireland) on adult WT and AROM+ female mice.

WT and AROM+ female mice (FVB/N; 24-27 weeks old; n= 6-8/grp) received a single s.c. injection of letrozole at 1mg/kg body weight daily for 6 weeks. Mice were subjected to daily vaginal smears. Controls were killed during post-estrus, while the treated groups were killed at the end of treatment period. The ovaries, uterine horns and gonadal fat were collected and weighed. One ovary and the uterine horns were fixed in formalin for histological assessment, while the other ovary was snap frozen in Ultraspec solution for RNA isolation. Serum was collected for hormone measurements.

AROM+ mice exhibited an abnormal cycle that alternated between estrus and post-estrus. Letrozole restored normal estrous cycles in AROM+ female mice, but did not alter body, ovarian or uterine weights. There were no differences in estrogen receptor alpha (ERα) or ERß mRNA expression by the ovary after letrozole treatment. Letrozole decreased WT and AROM+ female mice gonadal fat pad weight and adipocyte number when compared to the control WT. Histologically, hemorrhagic cystic follicles were occasionally present in both treated AROM+ and WT female mouse ovaries, whereas the uterine horn was appeared normal.

In summary, letrozole treatment restored normal estrous cycles in AROM+ female mice. The effect of letrozole on aromatase activity remains to be established and the serum estradiol analyses are in progress. Our studies demonstrated that excessive aromatase can lead to deleterious effects on the estrous cycle.

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IS NEUROGENESIS INVOLVED IN THE ENDOCRINE RESPONSE OF EWES TO RAMS?

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Exposure of anovulatory ewes to rams stimulates an increase in pulsatile LH in minutes and ovulation within days [1]. Traditionally, it was thought that ewes had to be isolated from rams before this endocrine response could be elicited, but more recent work indicates that isolation is not necessary if the ram is unfamiliar or 'novel'. Thus it appears that ewes can discriminate between individual rams and tailor their endocrine response accordingly. In other species, the rate of neurogenesis in the female brain is increased by exposure to males [2]. Therefore, we tested whether exposure to rams would stimulate an increase in neurogenesis in the hippocampus of ewes. During November, adult Merino ewes were allocated to one of two treatment groups; Ram (n=4) or Control (n=4). On Day 0, blood was sampled every 12 minutes for 6 hours before and 6 hours after ram introduction to the Ram ewes. Control ewes were sampled at the same frequency but remained isolated from rams throughout. Ewes were administered with the thymidine analogue, bromodeoxyuridine (BrdU; 100 mg/kg) immediately before ram introduction and at a parallel time in Control ewes. On Day 2, ewes were killed, decapitated and the heads perfused with 0.9% saline and 0.4% paraformaldehyde. The brains were removed and tissue containing the hippocampus was dissected out, frozen and maintained at -80°C until sectioning and processing for fluorescence immunohistochemistry. Ram introduction stimulated an increase in LH concentrations (0.33 ± 0.1l versus 1.31 ± 0.21 ng/mL) that was not observed in Control ewes (0.29 ± 0.23 versus 0.15 ± 0.12 ng/mL). Ram ewes had more BrdU labelled cells in the dentate gyrus of the hippocampus than Control ewes (8.28 ± 1.35 versus 6.12 ± 1.19 cells/mm^2) indicating a greater rate of neurogenesis in the ram-exposed ewes. It appears that memory formation driven by neurogenesis is involved in the 'ram effect'.

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REGIONAL EXPRESSION OF KISSPEPTIN, OESTROGEN RECEPTOR ALPHA AND GNRH MRNA WITHIN THE HYPOTHALAMUS OF THE COW

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Kisspeptin the product of the KISS1 gene and 17 b -oestradiol (OE) are important regulators of GnRH secretion. Kisspeptin is produced within the hypothalamus and acts directly on GnRH neurons through the receptor GPR54 whilst OE is of ovarian origin and appears to regulate GnRH through several mechanisms including binding to OE receptor-α (OERα). The aim in the present study was to determine the regional expression of KISS1, OERα and GnRH in the hypothalamus of the cow. Brains were obtained from 6 Brahman (*Bos* indicus) cows on Day 26 postpartum and a medial sagittal section was used to reveal the hypothalamus which was subdivided as follows: H1, anterior hypothalamic-preoptic area; H2, dorsal posterior hypothalamus; H3, ventral posterior hypothalamus including the mammillary body. H1 and H3 contained the anterior and posterior portions of the medial basal hypothalamus, respectively. Gene expression was determined by realtime PCR using tailored primers and the normalised expression data were analysed by one-way ANOVA followed by Bonferroni's multiple comparison test; results are means ± SEM. KISS1 was expressed predominantly ($P < 0.05$) in H3 (201.5 ± 99.9) whilst expression between H1 (9.8 ± 7.0) and H2 (0.2 ± 0.1) did not differ significantly. The highest ($P < 0.05$) expression of OERα was in H3 (215.7 ± 65.8) and expression between H1 (67.9 ± 24.0) and H2 (5.7 ± 3.9) did not differ significantly. GnRH expression was highest in H1 (25.4 ± 16.4) with similar expression in H2 (0.3 ± 0.1) and H3 (0.6 ± 0.2), although there were no significant differences between regions. The results indicated that KISS1, OERα and GnRH were predominantly expressed in either H1 or H3. KISS1 and OERα were expressed primarily in H3 whilst the highest expression of GnRH was in H1. All cows had yet to resume ovulation after calving and it could be suggested that the regional expression of KISS1, OERα and GnRH was reflective of cows not undergoing regular oestrous cycles.

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DEVELOPMENT OF AN IMPROVED E-SCREEN

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The existing E-screen was developed to measure estrogenicity of environmental pollutants. The proliferative effect of estrogens on MCF7 cells as an assay end point has not been standardised for water industry testing and the cells are difficult to maintain to high passage number. A comparative study of 5 cell lines, reported as expressing estrogen receptor alpha (ESR1), was conducted to improve the E-screen. An MTT assay was used to determine that the attachment time and optimum seeding density in a 96 well plate format were 4 hours and 20000 cells/well for the cell lines ZR75-1, Ovcar, RL-95, H23, T47D and MCF7. Three replicates of the assay showed ZR75-1 (3.00%) and T47D (3.67%) to have a lower inter-assay coefficient of variation than MCF7 (12.87%) when seeded at 20 000 cells/well for 72 hours. Conversely, the intra-assay coefficient of variation for the same conditions was high for T47D (41.02%) and ZR75-1 (29.18%) but low for MCF7 (8.33%). Based on total cell number at 72 hours, RL95-2 ($8.6x10^5$ cells) had the highest proliferation, T47D ($5.8X10^5$ cells) and ZR75-1 ($4.0x10^5$cells) had the lowest proliferation. Phenol Red has been reported as being estrogenic, but had no significant proliferative effect ($p>0.05$) when cells were cultured for 72h in 1% dextran-charcoal treated (DC) serum. Minimal growth was maintained in 1% DC serum. Western blotting confirmed the presence of the 66kDa ESR1 in ZR75-1, T47D and MCF7 but not Ovcar, H23 and RL95 cells. Quantitative RT-PCR was used to compare expression of ESR1 mRNA across the cell lines. MCF7 had the highest level (100%) followed by T47D (97.76%), ZR75 (85.75%) and Ovcar (67.15%). ESR1 mRNA was not detected in H23 and RL95 consistent with Western Blotting results. These initial data suggest that MCF7 and T47D are suitable candidates for developing an improved E-screen, although additional information regarding Estrogen Receptor beta (ESR2) and estrogenic responsiveness will also be taken into account.

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IN VIVO EFFECTS OF IMMUNIZATION AGAINST LEPTIN ON OVARIAN FUNCTION IN PRE-PUBERTAL FEMALE MICE

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Leptin has a regulatory role within the rodent reproductive axis as shown by the leptin deficient ob/ob mice, which apart from being obese suffer from hypogonadism and sterility. Leptin and leptin receptor mRNA has been found to be expressed in both human and mouse ovary as well as the endometrium. Leptin has been shown to modulate steroidogenesis by cultured ovarian somatic cells in a number of species and is mandatory for the embryonic implantation process in rodents.

The aim of this experiment was to investigate the role of leptin on ovarian and uterine function by studying the effects of passive immunization against leptin in prepubertal female mice (4 weeks old). The antibody (JMCK#43) used was raised in chickens against bovine leptin and purified IgY was used in the experiments. The mice were randomly divided into 4 treatment groups of 15 animals; antibody (50ug) with or without PMSG (1iu), and non immune antibody (50ug) with or without PMSG (1iu). The mice received daily injections for 4 days and were killed on the fifth day and the ovaries and uterus dissected out and weighed. The results of this experiment showed that the paired ovarian weight (12.0 ± 0.5 mg) in those mice treated with the antileptin IgY were significantly

heavier than those treated with non immune IgY (9.8 ± 0.6mg). PMSG significantly increased ovarian weight (15.9 ± 0.7mg) over both the control and antileptin treated animals. Interestingly, the combination of antileptin and PMSG significantly increased ovarian weight (21.3 ± 0.8) over all other treatment groups.

Although the ob/ob mouse demonstrates that an absolute absence of leptin results in infertility, this data suggests that peripheral leptin may act as an inhibitor of follicular development. The mechanism of action as yet remains unclear and further work is underway.

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THE ROLE OF IL-6 IN CAUSES SPREADING OF ENDOTHELIAL CELL DYSFUNCTION PREECLAMPSIA.

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Background: Preeclampsia is characterized by s ystemic maternal endothelial dysfunction. The exact cause of this dysfunction is unknown but it is induced by a placental factor. Our previous data shows that the activation of endothelial cells was induced after endothelial cell phagocytosis of necrotic trophoblast . We undertook this study to further investigate whether soluble factors are produced by endothelial cells after phagocytosing necrotic trophoblasts that could activate additional endothelial cells.

Methods: endothelial cells (HMEC-1) were cultured with either necrotic or apoptotic Jar cells for 24 hours. The conditioned media (CM) from these HMEC-1 was transferred to fresh HMEC-1. Activation of the fresh HMEC-1 was determined by cell-based ELISA for E-selectin and ICAM-1. The levels of cytokines including, interleukin-6 (IL-6), IL-1 b in the CM was measured by ELISA.

Results: The CM from endothelial cells that had phagocytosed necrotic but not apoptotic Jars activated fresh monolayers of HMEC-1. The level of IL-6 was elevated in CM from HMEC-1 that had phagocytosed necrotic, but not apoptotic Jars. Furthermore, the activation of endothelial cell which induced by CM was blocked by IL-6 antibody.

Conclusion: This study suggests that endothelial cell activated following phagocytosis of necrotic trophoblasts secrete IL-6 which in turn could activate additional endothelial cells which may not have been in direct contact with the necrotic trophoblasts. These findings suggest a mechanism whereby pulmonary endothelial cells activated after phagocytosing necrotic syncytial knots could induce the activation of bystander endothelial cells in other vascular beds. This may contribute to the systemic endothelial cell dysfunction seen in preeclampsia.

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HOMEOBOX GENE *HEX* EXPRESSION IS INCREASED IN HUMAN IDIOPATHIC FETAL GROWTH RESTRICTION.

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Angiogenesis is a fundamental aspect of normal placental development and reduced angiogenesis, particularly in the microvasculature, is frequently associated with common placental pathologies including fetal growth restriction (FGR) (1). In the placenta, as is the case in embryo and adult tissues, angiogenesis and vascular development are regulated by Homeobox gene transcription factors. We have recently detected the homeobox gene *HEX* (Hematopoietically EXpressed homeobox) expression in placental microvascular endothelial cells (Murthi et al. unpublished Data). *HEX* is a negative regulator of endothelial cell function and decreases the expression of angiogenesis-related genes in cardiovascular development (2). Here, we test the hypothesis that *HEX* expression is increased in idiopathic FGR. Placentae from pregnancies complicated by idiopathic FGR (n=25) and from gestation age-matched controls (n=25) were collected, and the level of *HEX* mRNA was determined using real-time PCR as described previously (3). Relative quantitation of *HEX* mRNA normalized to the house-keeping gene *GAPDH* demonstrated a significant increase in *HEX* expression in FGR [1.38 ±0.27, FGR (n=25) vs. 1.08±0.21, control (n=25), t-test, $p<0.03$] compared to gestation-matched controls. We conclude that increased expression of *HEX* in FGR-affected placentae is consistent with an anti-angiogenic role and may contribute to the aberrant angiogenesis seen in this pregnancy disorder.

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SYNTHESIS OF TUMOR NECROSIS FACTOR-RELATED APOPTOSIS-INDUCING LIGAND (TRAIL) BY EXTRAVILLOUS TROPHOBLAST IS REGULATED BY P38 MITOGEN-ACTIVATED PROTEIN KINASE AND JANUS KINASE 1

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During human pregnancy, two physiological roles for tumor necrosis factor-retated apoptosis inducing ligand (TRAIL) have been identified. We have demonstrated that TRAIL is used by fetal trophoblast in the remodelling of uterine spiral arteries[1]. It is also important in the maintenance of maternal fetal immune tolerance. Defects in TRAIL production may compromise these essential functions and potentially contribute to pregnancy complications such as pre-eclampsia. The cellular effects of TRAIL have been extensively investigated however pathways leading to its synthesis are not well characterized. We examined pathways regulating TRAIL synthesis in the extravillous trophoblast-derived cell line SGHPL-4, using ELISA to measure TRAIL production. We tested the ability of epidermal growth factor, transforming growth factor β, corticotrophin releasing factor, human chorionic gonadotrophin, insulin-like growth factor-II, interleukin-10, interferon γ (IFNγ), interleukin-1β and tumor necrosis factor α (TNFα) to stimulate TRAIL production. These ligands are all produced or up-regulated at the fetal-maternal interface during pregnancy.

IFNγ was the only factor that caused a significant, concentration-dependent induction of TRAIL synthesis. IFNγ, in combination with TNFα, stimulated a time-dependent increase in TRAIL production over 24 hours (significant at 6 hours). Inhibition of p38 mitogen-activated protein kinase (p38mapk) with SB203580 or SKF86002 abrogated IFNγ/TNFα-stimulated TRAIL synthesis. Inhibition of Janus kinase 1 (Jak1) activation also blocked TRAIL production.

We conclude that TRAIL production in extravillous trophoblast is selectively regulated and, in response to IFNγ, is dependent on activation of the p38mapk and Jak pathways.

(1) RJ Keogh et al. 2007 Circ Res 100:834-41

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THE EFFECT OF OXYGEN AND TUMOR NECROSIS FACTOR ON THE NUMBER OF TROPHOBLASTS SHED FROM THE HUMAN PLACENTA *IN VITRO*

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The placenta in viviparous animals represents a tissue transplant since the fetus and placenta are genetically and immunologically derived in part from the father. The shedding and deportation of cells called trophoblasts from the placenta into the maternal blood is a feature of normal human pregnancy, during which there are up to 3 x 10^6 "immunologically foreign" trophoblasts shed into the maternal peripheral circulation daily. Therefore, it seems intuitively likely that they contribute to the normal maternal physiological adaptation to pregnancy . Increased trophoblast shedding is seen in preeclampsia, one of the most common diseases of pregnancy, and presently the reasons for this increased shedding are not known. Decreased placental oxygenation, increased oxidative stress and high levels of pro-inflammatory cytokines such as TNF-α have been implicated in the pathophysiology of preeclampsia and there is some evidence that the formation of syncytial knots (a type of shed multinucleated trophoblast) may be increased by exposure to these factors. In order to test this hypothesis using an *in vitro* model of trophoblast deportation, ten first trimester placentae were cultured for 72 hours in atmospheres containing 1%, 8% or 20% oxygen. Similarly, first trimester placentae were also cultured with or without 10ng/ml TNF-α and 10ng/ml IFN-γ. The number of syncytial knots, mononuclear trophoblasts and trophoblast "ghosts" that were shed from the explants was not significantly different between the various oxygen conditions. After 24 hours of culture the number of shed trophoblasts were similar between the no treatment and cytokine treatment groups, but following a further 48 hours of culture the explants that had been exposed to the cytokines showed a three fold increase in trophoblast shedding.

Our data suggest that hypoxia does not increase trophoblast shedding *in vitro* from first trimester placentae, but that the pro-inflammatory cytokines TNF-α and IFN-γ do increase trophoblast shedding and therefore may play a more important role in the pathogenesis of preeclampsia than decreased placental oxygenation.

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FOLATE NUTRIGENOMICS AND MATERNAL DNA DAMAGE IN UTEROPLACENTAL INSUFFICIENCY

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A variety of potentially life-threatening and common pregnancy complications are associated with uteroplacental insufficiency (UPI), including preeclampsia (PE) and intrauterine growth restriction (IUGR). Currently, we cannot predict which women will develop these complications. We conducted a prospective observational study to compare genome damage markers and circulating maternal concentrations of folate, vitamin-B12, vitamin-B6 and homocysteine in women with healthy pregnancies and those who develop

UPI, PE and IUGR. At 20 weeks gestation maternal blood was collected in order to measure genome damage (micronuclei, nucleoplasmic bridges, nuclear buds) and cytotoxicity (apoptosis, necrosis, nuclear division index) markers in peripheral blood lymphocytes using the cytokenesis block micronucleus assay. Circulating concentrations of folate, vitamin-B12, vitamin-B6 and homocysteine were also measured and compared in 50 healthy pregnant women with normal outcomes and in 93 women with high risk pregnancies. UPI was defined as PE or gestational hypertension, and/or IUGR (<10th centile) and/or placental abruption. Comparisons of differences in the genome damage markers and circulating concentrations of micronutrients and homocysteine were made between data from pregnancies with healthy outcomes and data from those complicated by UPI , PE and IUGR using independent t-tests and logistic regression to control for confounding factors such as age, smoking and BMI. Micronucleus frequency (%) was significantly increased in women who developed UPI, PE and IUGR (controls: 15.8 %, UPI: 24.1 %, P=0.002; PE: 29.9 %, P=0.005; IUGR 24.7 %, P=0.023). Red cell folate was significantly reduced in women with IUGR (controls: 669.2 nmol/L, IUGR 499.6 nmol/L, P=0.018). Plasma homocysteine was also increased in women who developed UPI and IUGR (controls 4.3 μmol/L, UPI 5.2 μmol/L P=0.027; IUGR 5.7 μmol/L, P=0.016) despite high supplementation with folic acid, vitamin-B12 and vitamin-B6. Our study supports the hypothesis that increased micronucleus frequency in maternal peripheral blood lymphocytes and high homocysteine at 20 weeks gestation may have useful predictive value for the development of UPI including preeclampsia and IUGR.

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THE EFFECTS OF NF-KB INHIBITORS ON PRO-INFLAMMATORY CYTOKINE GENE EXPRESSION AND APOPTOSIS IN HUMAN CHORIODECIDUAL CELLS

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Inflammatory activation of fetal membranes plays a central role in the pathogenesis of preterm labour. Nuclear factor-kB (NF-kB) is a transcription factor activated by inflammatory cytokines and Toll-like receptors that serves as a nexus for inflammatory signalling, and hence is of interest as a target for anti-inflammatory pharmacologics. We have tested a number of commercially-available small molecule NF-kB inhibitors for their ability to inhibit intrauterine LPS-stimulated cytokine production and inflammatory gene expression *in vitro*. Choriodecidual cells were isolated from term extraplacental membranes by collagenase / dispase digestion and cultured in 24- and 6-well plates for 24-48 h before addition of LPS (100 ng/ml) and test substances at a range of concentrations. Media (at ≥20 h) was collected and analysed for IL-6 and TNF-a content by ELISA and Luminex assay, respectively. Total RNA was extracted after 4 h treatment and used to probe oligonucleotide arrays (Superarray) containing 112 inflammation-associated genes. Nuclear proteins were extracted (3, 6 and 16 h), isolated and examined for NF-kB content (p65) by immunoblotting. Cell viability/apoptosis was determined by MTT assay and M30 antigen (a caspase-3 substrate) staining. Of the nine inhibitors tested, caffeic acid phenethyl ester (CAPE), IKK2 inhibitor IV (IKK2inh), parthenolide (Pth) and helanalin (Hln) inhibited LPS-induced cytokine production at concentration ≥ 0.2 mM, with IKK2inh being the most potent. Production of IL-6 and TNF-a was reduced by >90 % at the highest doses tested, with little or no evidence of apoptosis. A large number of inflammation-associated genes up-regulated by LPS treatment were inhibited by all four compounds, including some associated with inhibition of apoptosis. Nuclear translocation of p65/RelA, a hallmark of inflammatory activation, was abrogated by IKK2inh, Pth and Hln. These findings have identified several anti-inflammatory compounds that may be candidate drugs for use in the prevention of preterm labour associated with an inflammatory aetiology.

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PLACENTAL PRODUCTION AND SECRETION PROFILES OF THE NEWLY IDENTIFIED SERINE PROTEASE HTRA3 THROUGHOUT HUMAN PREGNANCY AND ASSOCIATION WITH PLACENTAL INSUFFICIENCY

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HtrA3 is a newly identified serine protease [1] that is up-regulated at the maternal-fetal interface during early pregnancy [2] . HtrA3 protein is detectable in human pregnancy serum [2] . The present study aimed to establish the placental expression pattern of HtrA3 and its serum profile at different times of gestation, and to determine whether HtrA3 profiles differ between normal pregnancies and those complicated by placental insufficiency in women. HtrA3 mRNA expression was determined by RT-PCR and HtrA3 protein levels and cellular localization were determined by immunohistochemistry, in placental tissues from 1st, 2nd, and 3rd trimester (n=5/stage/group) of normal pregnancy and from pregnancies in which placental insufficiency occurred. HtrA3 levels in maternal blood were evaluated by Western blotting of serum samples from women at different gestational ages (n=10-20/stage/group). Placental HtrA3 expression was highest in the 1st-trimester, with HtrA3 protein being localized strongly in villous syncytiotrophoblast, trophoblast cell columns, trophoblast shell, and endovascular trophoblast. HtrA3 expression was much lower in the 2nd trimester and term placenta with the protein localized primarily in the syncytiotrophoblast. Serum HtrA3 levels were maximal in the 1st trimester. HtrA3 profiles were not identical between normal pregnancies and those with placental insufficiency. These results indicate that HtrA3 is strongly associated with and potentially critical for placental development, and that placental HtrA3 production is reflected by its levels in maternal serum.

(1) Nie et al. 2003, Biochem J, 371:39-48

(2) Nie et al. 2006, Biol Reprod, 74:366-374

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A COMPARISON OF GENE EXPRESSIONS BETWEEN FIRST TRIMESTER, TERM AND PREECLAMPTIC HUMAN PLACENTAE

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The human placenta is vital for gas, nutrient and waste exchange and the maintenance of pregnancy; however the genes driving trophoblast function are poorly understood. We examined the expression of several genes, including those involved in hormone signalling (β-hCG) and endothelial functioning (eNOS), and the hitherto uncharacterised in the human placenta caspase-14. Gene expression levels were examined between first trimester, term and preeclamptic human placentas using quantitative Real Time PCR. Preeclampsia is a maternally manifested disorder of pregnancy with unknown aetiology. One hypothesis for its presence involves abnormal trophoblast differentiation leading to increased syncytial shedding by apoptosis into the maternal circulation. In accordance with the established dogma, β-hCG transcription was significantly higher in first trimester placentae than at term ($P<0.001$), indicating β-hCG production is greatly reduced with gestational age. Beta-hCG mRNA was not differentially expressed in preeclampsia. As a modulator of nitric oxide signalling, eNOS also exhibited substantially reduced transcription across gestation ($P<0.05$), indicating an endothelial role for the villous trophoblast during placental invasion and oxygen tension modulation. Immunohistochemistry revealed caspase-14 to be isolated to the trophoblast of the chorionic villi, however no changes in caspase-14 transcription were noted between human first trimester, term and preeclamptic placenta specimens. The molecular profile of the placenta is dynamic across gestation; however none of the genes examined presented an altered pattern with preeclampsia. The observed alterations represent the dynamic changes in trophoblast physiology and function that occur with increased gestational age.

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RISING FSH IN AGEING TRANSGENIC FSH MICE ACCELERATES FEMALE REPRODUCTIVE FAILURE: OOCYTE, EMBRYO AND/OR UTERUS?

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Rising serum follicle-stimulating hormone (FSH) is one of the earliest signs of reproductive ageing in women, coinciding with declining fecundity several years before menopause, and is considered to reflect ovarian follicle depletion. Whether or not elevated FSH directly contributes to age-related declining fecundity has received little investigation, largely due to lack of specific *in vivo* models. We generated transgenic (Tg) mice with rising serum human FSH, independent of follicle depletion, that produced larger litter sizes <20 weeks of age, then rapidly declining litter size from 20-40 weeks old (wo) culminating in premature infertility[1]. Despite declining fertility, we show that increased ovulation was maintained in fertile 9 wo (2.9-fold, $p<0.001$) and then subfertile 23 wo (2.8-fold, $p<0.0001$) TgFSH females relative to fertile age-matched wt females. However, aging TgFSH females appear to exhibit mating behaviour changes with an increased percentage of older TgFSH compared to wt females (78% vs. 46%) mating on the first night paired with a male, and more first night mated TgFSH females failing to ovulate compared to controls (57% vs. 33%). Furthermore, analysis of pregnant TgFSH females at 21 days post coitum (dpc) revealed a significant rise in embryo-fetal resorption (up to 7-fold, $p<0.05$) and parturition failure with age compared to wt females. Preliminary results from embryo transfer experiments to dissect age-related effects of rising TgFSH on embryo quality or uterine function reveal that embryos (8-10/unilateral oviduct transfer) from old TgFSH females develop normally in young wt recipients by 19 dpc, indicating embryo quality is not a major contributor to infertility of aging TgFSH females. Conversely, most old TgFSH recipients with young wt embryos failed to give birth by 19 dpc, however pups in utero appear normal without excessive embryo-fetal resorption, suggesting complex temporal ovarian-uterine effects contribute to declining fertility. This initial data demonstrates that the TgFSH female mice provide a novel paradigm to selectively determine effects of rising FSH, independent of follicle depletion, on the age-related decline in female fertility.

(1) McTavish KJ, et al. Endocrinology. 2007 May 31; [Epub]

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EFFECT OF IGF-II, UPA AND PLASMINOGEN IN COMBINATION ON BLASTOCYST DEVELOPMENT

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The expression of Insulin-Like Growth Factor-II (IGF-II) is delayed and reduced in IVF mouse embryos while that of urokinase Plasminogen Activator (uPA) is reduced in cultured rat embryos (Stojanov et al. 1999; Aflalo et al. 2005). Interestingly, t he addition of Plasminogen or IGF-II to culture media improves development to the blastocyst stage in murine embryos (Menino & O'Claray, 1986; Harvey & Kaye, 1992) and Plasminogen improves hatching rates in ovine embryos (Menino et al. 1989), suggesting a combination of factors may counteract the negative effects of ART. We have discovered an important interaction between IGF-II, Plasminogen and uPA and aimed to determine whether these, in combination, improve *in vitro* embryo development. Zygotes were collected from 21 day old superovulated C57Bl6 female mice and cultured in groups of 10 in 50 ul drops of BlastAssist Medium 1 (Medicult). The next day, 2-cell embryos were divided between treatment groups, either Medium 1 alone (controls), or with 12.5nM IGF-II or 12.5nM IGF-II, 5 mg/ml uPA and 10 mg/ml Plasminogen. Embryos were assessed 24 h later and transferred to BlastAssist Medium 2 with the appropriate treatment for a further 48 h. Treatment effects were assessed using univariate ANOVA with replicate as a covariate (N=9-10 replicates/group). Unlike previous reports, exposure to IGF-II did not increase the proportion of embryos reaching the blastocyst stage of development (71.8%), compared to controls (68.8%). Similarly, treatment with IGF-II, Plasminogen

and uPA in combination did not significantly increase development to blastocyst (80.8%). However, the combination treatment tended to increase the proportion of blastocysts hatching at 96 h of culture (46.4%), compared to controls (34.1%) and this increase was significant when compared to culture with IGF-II alone (31.6%, p=0.05). Further replicates and embryo transfer experiments are currently underway to determine if the combination of IGF-II, uPA and Plasminogen improves implantation rates, placental development and pregnancy outcome.

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(5) Menino et al. Biol Reprod, 1989. 41(5): 899-905

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INSIGHTS INTO GROWTH HORMONE ACTION FROM MOUSE MODELS

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Processes such as postnatal growth and metabolism can only be studied effectively in animal or human models, and these have recently provided an understanding of the signalling pathways driving GH-dependent IGF-1 production, hence postnatal growth. It is now established that STAT5b activation by JAK2 is a key requirement for such growth, based on STAT5b deletion in humans and in mice, as well as targeted deletion of STAT5 activation domains in the GH receptor. Suppression of STAT5a/b signalling by GH also results in increased adiposity in mature male mice, and microarray studies in these mice have identified a number of key metabolic genes whose expression is regulated by STAT5. Indeed, STAT5b signalling is the basis for a substantial amount of sexual dimorphism in hepatic gene expression. However, JAK2/STAT5 signalling is not the only pathway utilised by the GH receptor for regulation of gene expression. We have shown in vitro that the GH receptor can activate src kinases independently of JAK2, and now report that mice with targeted mutation of the JAK2 binding sequence of the receptor (Box1) are able to activate src/ras/ERK in the absence of JAK2/STAT5 activation. Moreover, microarray analyses on the livers of these mice have identified transcripts regulated by src family kinases, including a number known to be regulated by ERK. This observation explains a number of puzzling clinical reports of independent regulation of ERK and STAT5 in cases of growth impairment. Tandem tyrosine kinase usage may also may be typical of a number of other class 1 cytokine receptors such as prolactin and EPO receptors. Supported by NHMRC grants to MJW.

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IGF SIGNALING THROUGH AN IGF RECEPTOR-BOUND TRANSCRIPTION FACTOR

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The type I insulin-like growth factor receptor (IGF-I receptor) activates multiple intracellular pathways that mediate effects of IGFs on cell proliferation, differentiation, apoptosis, and survival. These responses occur in part from modulation of gene expression as a result of activation or inhibition of specific transcription factors. All IGF-responsive transcription factors thus far characterized are regulated by signaling reactions several steps downstream from the receptor. We now have identified Y-box binding protein 1 (YB-1) as the first example of an IGF-I responsive transcription factor that interacts directly with the IGF-I receptor. Previous yeast 2-hybrid cloning studies have demonstrated strong binding of multiple SH2-domain containing proteins to the intracellular portion of the IGF-I receptor. Since rapid growth of these clones could mask the presence of other interactive proteins, we used the yeast 2-hybrid method to screen a mouse embryonic cDNA expression library with an intracellular region fragment of the IGF-I receptor modified to exclude the C-terminal SH2-binding region. Multiple positive clones were obtained corresponding to members of the Y-box protein family, with the majority of clones encoding the YB-1 transcription/translation regulatory protein. To further investigate the interaction of YB-1 with the IGF-I receptor, a GST-YB-1 fusion protein was generated, and this was shown to precipitate the IGF-I receptor from extracts of cultured fibroblasts expressing the IGF-I receptor. Co-precipitation of the IGF-I receptor and YB-1 was evident in the basal state and decreased significantly after IGF-I treatment. When NIH-3T3 fibroblasts were stimulated with IGF-I, a tagged YB-1 construct was shown by quantitative immunocytochemistry to shift from cytoplasmic to nuclear localization with a peak effect at 2-3 hours. Similarly, YB-1 detected by quantitative immunoblotting was shown to increase more than 2-fold in nuclear subfractions of NIH-3T3 cells following IGF-I stimulation. Previous studies have shown that IGF-I stimulates PTP-1B expression in L6 skeletal muscle myotubes. We found that IGF-I treatment of L6 myoblasts resulted in 2-fold activation of a luciferase reporter linked to a portion of the human PTP-1B enhancer and endogenous promoter. The PTP-1B promoter binds YB-1 and, in initial experiments, the effect of IGF-I on the PTP-1B-luciferase reporter in L6 myoblasts was inhibited by YB-1 anti-sense. Subsequent experiments have shown that YB-1 siRNA results in a decrease in both YB-1 and PTP-1B mRNA by quantitative PCR in L6 cells. The binding of YB-1 to the IGF-I receptor represents the first demonstration of a direct interaction between a transcriptional regulator and the IGF-I receptor. These data support a novel pathway in which IGF-I receptor activation results in YB-1 dissociation from the receptor, localization to the nucleus, and activation of gene transcription. In addition to likely effects on other YB-1 regulated genes, we speculate that YB-1 stimulation of PTP-1B transcription may function in the down-regulation of IGF-I signaling by promoting IGF-I receptor dephosphorylation.

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ONCOGENIC POTENTIAL OF AUTOCRINE HUMAN GROWTH HORMONE

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The hGH gene is expressed in epithelial cells of the normal human mammary gland. Increased epithelial expression of the hGH gene is associated with the acquisition of pathological proliferation, and the highest level of hGH gene expression is observed in metastatic mammary carcinoma cells. Autocrine hGH production in human mammary carcinoma cells results in increased cell proliferation and survival associated with alterations in morphology. We have further demonstrated that autocrine production of hGH in immortalized human mammary epithelial cells concomitantly enhances proliferation and offers protection from apoptosis; forming the basis for abnormal mammary acinar morphogenesis, oncogenic transformation and tumor formation in vivo. Thus, simple forced expression the hGH gene is sufficient for oncogenic transformation of the immortalized human mammary epithelial cell. Moreover, autocrine production of hGH, in mammary carcinoma cells with epithelial morphology, promotes mesenchymal cellular morphology, increased cell migration and increased metalloprotease (MMP) activity with subsequent acquisition of invasive behavior both in vitro and in vivo. In stark contrast to the oncogenic and metastatic potential of autocrine hGH, exogenous hGH neither supports tumor formation nor invasion by human mammary epithelial cells. We have utilized this discrepancy in the oncogenicity of autocrine and exogenous hGH in an attempt to identify molecules which could potentially be involved in oncogenic transformation of the human mammary epithelial cell. We were able to extract a subset of 305 genes which were remarkably different in their response to autocrine and exogenous hGH. Functional analysis of two of the identified autocrine hGH regulated genes, trefoil factors 1 and 3 (TFF1/3), determined that these soluble factors mediated the oncogenic effects of autocrine hGH in human mammary carcinoma cells. We therefore postulate that autocrine hGH functions as a higher order switch, controlling at least some of the genetic elements required for oncogenic transformation and neoplastic progression of the human mammary epithelial cell.

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CELL SIGNALLING BY IGFBP-3: HOW DO YOU DO WHAT YOU DO TO ME?

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Insulin-like growth factor binding protein-3 (IGFBP-3) is a multifunctional protein that has a key role in normal growth and metabolism as a carrier of the insulin-like growth factors (IGFs) in the endocrine system. IGFBP-3 is equally important in regulating IGF bioactivity in the pericellular environment; in all cell types examined so far, a molar excess of exogenous IGFBP-3 can abolish ligand-mediated activation of the signaling type 1 IGF receptor (IGFR1) by sequestering IGF-I or IGF-II, resulting in a loss of their ability to stimulate cell proliferation and survival. IGFBP-3 also influences cellular growth and survival independently of its binding these ligands, with both growth-inhibitory and -stimulatory activity observed *in vitro*. The mechanisms underlying these "IGF-independent" actions appear complex, and observations of exogenous and endogenous IGFBP-3 failing to elicit the same cellular effects suggest that it functions differently in the intracellular and extracellular environments. Intracellularly, the effects of IGFBP-3 may be mediated by changes in the expression or activation of cell cycle proteins, apoptotic effectors and signaling pathway intermediates, and interaction with intracellular proteins and nuclear receptors. The effects of extracellular IGFBP-3, apart from those involving binding of IGFs, are not well understood mechanistically. The existence of a signal-transducing, cell-surface IGFBP-3 receptor was proposed over 15 years ago but such a protein has not yet been identified. Our data indicate an alternate mechanism of action of IGFBP-3 by which it can modulate the activity of other growth factor signalling pathways initiated from outside the cell. We have shown that in breast epithelial cells IGFBP-3 potentiates EGF-stimulated activation of the EGF receptor, resulting in increased activation of growth-regulatory pathways downstream of the receptor, such as the p44/42 and p38 MAPK pathways, and enhancement of DNA synthesis and cell proliferation. IGFBP-3 also potentiates IGF signalling mediated by IGFR1; significantly, this does not involve direct interaction of IGFBP-3 with the growth factor as it can be reproduced using an IGF analog that does not bind to IGFBP-3. Our most recent data now indicate that the effects of IGFBP-3 on EGF- and IGF-stimulated growth can be blocked by inhibitors of sphingosine kinase (SK) activity, implicating the product of SK action, sphingosine-1-phosphate, in the potentiating effect of IGFBP-3 on these growth factor-activated pathways.

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CUTTING OUT THE MIDDLE-MAN - AUTOCRINE INHIBITION OF VASOPRESSIN SECRETION BY CENTRAL NEUROPEPTIDE RELEASE

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Vasopressin (antidiuretic hormone) is secreted in response to increased plasma osmolality or decreased blood volume to maintain plasma osmolality and blood pressure by promoting antidiuresis and vasoconstriction. Vasopressin secretion is determined by action potential (spike) discharge initiated within the hypothalamus and vasopressin neurons display a range of activity patterns: some neurons are silent, some irregular, some continuously active and some display rhythmic ‘phasic' activity, alternating between periods of activity (bursts) and silence that each last tens of seconds. Hence, the vasopressin concentration in the circulation reflects the average activity across the population of vasopressin neurons.

Kappa-opioid receptor antagonism increases intraburst firing rate and burst duration in phasic vasopressin neurons (1); this increase in firing rate is not evident at burst onset but emerges as bursts progress (2). By contrast kappa-opioid receptor antagonism, V1

vasopressin receptor antagonism increases firing rate throughout bursts (2). Hence, the actions of endogenous vasopressin are generally inhibitory and tonically present. The kappa-opioid peptide, dynorphin, is co-packaged in vasopressin neurosecretory vesicles and secreted from vasopressin neurons cell bodies and dendrites during periods of activity; our data indicate that this dendritically released dynorphin feeds back to inhibit vasopressin neurons, terminating activity to optimize vasopressin secretion from individual neurons at the posterior pituitary gland.

We have now found that kappa-opioid receptor antagonism also increases the activity of irregular vasopressin neurons, but not continuously active vasopressin neurons. Hence, continuously active vasopressin neurons might 'escape' the autocrine feedback inhibition that terminates activity. Thus, variability in the strength of autocrine kappa-opioid inhibition of vasopressin neurons allows vasopressin neurons to express a range of activities under basal conditions that will result in baseline vasopressin secretion from the posterior pituitary gland that can then be increased or decreased in response to the appropriate physiological stimuli.

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MOLECULES AND MONOGAMY: WHAT'S LOVE GOT TO DO WITH IT?

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Many aspects of social behavior, including what humans call "love," are affected by changes in neuropeptide systems regulated by oxytocin (OT) and arginine vasopressin (AVP). This presentation will be based primarily on data from socially monogamous prairie voles (Microtus ochrogaster), which share with humans a tendency to high levels of sociality, the capacity to form long-lasting social bonds, and to exhibit biparental care of young. In this species, the presence or absence of a social partner or brief exposure to an infant can alter levels of OT, AVP and CRF. Such manipulations also can affect neurogenesis, measures of heodonia/depression and cardiovascular function (Grippo, et al., Biol. Psychiat., 2007). Several effects of social isolation were reversed by exogenous OT. Changes in the behaviors used to define social monogamy also can be seen as a consequence of neonatal exposure to OT or AVP. In addition, these behaviors are sensitive to subtle changes in how offspring and parents are handled in early life. In the first week of life even a single manipulation - hormonal and/or social - can influence the later expression of the traits of social monogamy. The effects of both behavioral and endocrine manipulations are sexually-dimorphic, with long-lasting changes in the CNS expression of OT or AVP (V1a) receptors and OT or AVP, as well as measures of sociality. These findings may have implications for medical treatments that manipulate OT (such as Pitocin or OT antagonists) and for sexually-dimorphic developmental disorders such as autism (review Carter Behav. Brain Res. 2007).

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KEEPING A COOL HEAD: CNS INTEGRATION OF HORMONAL AND BEHAVIOURAL OSMOREGULATORY AND THERMOREGULATORY MECHANISMS

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When mammals become dehydrated, several homeostatic responses are engaged to counteract the loss of body water. These responses include vasopressin secretion from the neurohypophysis to minimise further fluid loss in urine and thirst to stimulate drinking and restore the fluid loss. Early studies from our laboratory identified brain osmoreceptors within the organum vasculosum of the lamina terminalis (OVLT) that play a crucial role in initiating these responses. Our more recent studies utilising a number of techniques have shown that neurons throughout the lamina terminalis (in subfornical organ and median preoptic nucleus as well as OVLT) exert osmoregulatory control over vasopressin secretion and thirst. We have also observed that this osmoregulatory region of the brain influences thermoregulation. When animals are exposed to a hot environment so that core body temperature increases, thermoregulatory responses such as sweating in humans and/or panting in animals result in evaporative cooling of the body. If the fluid losses from sweating and panting are not replaced, animals become dehydrated therefore compromising body fluid homeostasis. Our studies in sheep show that as the osmolality of body fluids increase, thermoregulatory panting is inhibited. Furthermore, central osmoreceptors that exert an inhibitory influence on panting responses appear to be located within the lamina terminalis. We speculate that osmoregulatory mechanisms within the lamina terminalis may influence thermoregulatory sweating in humans. The relationship between osmoreceptors regulating vasopressin secretion, thirst and panting responses remains to be determined.

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THE NEUROPHYSIOLOGY OF LOVE

R. V. Jackson, P. Hlincik, T. Sedgley, I. Clarke, K. McMahon, M. Jones, M. Griffin

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We have set out to investigate the hypothesis that the neruopeptides oxytocin(OT) and arginine vasopressin(AVP) are crucial central nervous system(CNS) mediators of human attachment, bonding, affiliation and love.

Extensive animal, non-human primate and limited human evidence exists that OT/AVP brain systems, interacting with dopamine reward circuits, are key mediators for building social bonds. Intranasal delivery, in humans, of OT and AVP has been shown to modulate trust, fear, aggression and befriending attitude. Functional MRI studies in humans demonstrate activation of brain areas rich in OT/AVP receptors and/or innervation and interconnected dopamine-reward areas when a beloved's photograph is shown to his/her partner. Using fMRI, photographs of a partner and friend, and psychological measures after intranasal administration of OT and/or AVP, we report pilot data in 8 male and 8 female volunteers, aged 18-53 years old, who have been in stable heterosexual relationships for > 6 months, and whose friend of the same sex as the partner has also been known for > 6 months. The volunteers had no medical or psychological conditions, were not smokers, nor did they have any abnormalities of the nasopharyngeal region. The studies were performed in a randomised, double-blind, placebo-controlled, factorial design consisting of 40 IU AVP or Placebo combined with 40 IU OT or Placebo delivered by intranasal spray over 5 to 8 minutes, followed by exposure to photographs of their partner and friend in a random manner during a 16 minute fMRI session. Before, during and after the fMRI task they were given psychological questionnaires or tasks and blood samples were taken for measurement of OT, AVP, HPA axis and HPG axis hormones.

These studies aim to:

1. Examine any reported changes in participants' perceptions of their relationships with their parrtner or friend and any evidence of a sexually dimorphic response.
2. Identify specific brain circuitry activated and if there is a sexually dimorphic response
3. Determine if there is a relationship between activated areas and psychological measures
4. Determine peripheral blood levels of OT, AVP, HPA axis and HPG axis hormones and if there is any relationship between these levels and activated brain areas.

Our overall aim is to shed light on the neurobiology of one of the most important psychoneuroendocrine functions in humans - the behavior required for attachment and affiliation to ensure good mental health and reproduction and preservation of the species; and, to develop therapeutic interventions to repair interpersonal relationships and to treat disorders characterised by dysfunctional social interactions, such as autism spectrum disorder, social phobia, post-traumatic stress disorder and borderline personality disorder.

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OVERVIEW OF THERAPEUTIC CLONING AND STEM CELL THERAPY - THE SCIENCE BEHIND THE HEADLINES

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The ability to create a source of stem cells from a patient and use these cells to replace diseased or damaged tissue – so called therapeutic cloning – has captured the world's attention. Lauded by some as a possible means of treating incurable diseases, condemned by others as the potential to create life for spare parts, what is often lost in the polarised coverage is a full understanding of the fascinating biology and the complex ethical and regulatory issues behind this aspect of stem cell science.

Therapeutic cloning simply involves the transfer of the nucleus of a patient's cell into the cytoplasm of an oocyte from which the nuclear material has been removed. Following artificial activation, the reconstituted oocyte undergoes blastomere cleavage culminating in blastocyst development from which stem cells can be obtained. As these stem cells would be genetically matched to the patient, they could theoretically be transplanted into the patient without the need for immunosuppression.

Underlying the mechanical events involved in therapeutic cloning is the remarkable reprogramming capacity of the egg cytoplasm that enables embryonic equivalence to be restored to a differentiated adult somatic cell. While the exact cytoplasmic factors and mechanisms involved in reprogramming remains unknown, recent animal studies have indicated that the ability to convert an adult cell to a stem cell may only involve four factors. It is the unravelling of the mystery of reprogramming that has captivated scientists for decades and once solved will truly revolutionise cell replacement therapy in humans.

However, accompanying this intriguing biology are significant ethical and social issues. Since the early days of IVF, the use of oocytes and embryos in research has caused public unease. Therapeutic cloning heightens these concerns by raising additional issues such as commodification of women as a source of oocytes, the status of the created embryo and whether allowing therapeutic cloning will lead to a slippery slope towards reproductive cloning. These are legitimate concerns which need to be address responsibly prior to conducting any experiments involving therapeutic cloning.

This talk will provide an insight into the topical field of therapeutic cloning, including the anticipated benefits and alternative technologies, from both a scientific and regulatory perspective.

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USE OF HUMAN EMBRYONIC STEM CELLS TO DEVELOP NOVEL THERAPIES FOR TYPE 1 DIABETES

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The incidence of type 1 diabetes is continuing to rise with at least 130,000 Australians with this disorder. Whilst administration of insulin subcutaneously is life saving and will lower blood glucose levels, the fine regulation of a ß cell cannot be achieved in this way. The only form of cell therapy for diabetes being practiced clinically at present is with human islets. Whilst beneficial to the few, their availability is quite limited, and their long term benefit uncertain. Stem cells are a potential source of ß cell surrogates, with both embryonic and adult sources being examined. Initial experiments with embryonic stem cells (ESC) tried to differentiate them

into ß cells via the ectodermal pathway of development. Few insulin-producing cells were made by this method with a number of features different from those of pancreatic ß cells. More recently attempts to differentiate ESC via an endodermal pathway commenced, the first step being exposure to activin A ± butyric acid. A series of subsequent steps using different reagents in the culture medium has resulted in production of insulin-containing cells, but there are some features of adult pancreatic ß cells missing. They are acute glucose-responsiveness and the absence of insulin-containing secretory granules. Other strategies being examined to produce ß cell surrogates from ESC include genetic manipulation, co-culturing with fetal pancreas and seeding the cells onto scaffolds.

Nuclear transfer is a new technology that eventually may be of benefit in developing patient-specific ESC lines which would not be rejected if transplanted into the recipient. However, whether such cells will need protection from autoimmune attack remains to be determined. If so, placing the cells inside microcapsules may be a way of providing protection from attack by these immune cells. A second potential benefit of nuclear transfer is to make an ESC line with diabetogenic genes such as DR3 and DR4 for the purpose of drug discovery.

Adult stem cells might also be of benefit in developing a cell therapy for type 1 diabetes. Sources of cells being examined for this purpose include the nose, liver, fetal and adult pancreas, cord blood, bone marrow and peripheral blood.

In summary, the creation of human ESC and their conversion into insulin-producing cells is accelerating the progress being made with cell therapies for diabetes, with clinical translation the ultimate goal.

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SOME SPEED BUMPS ON THE ROAD TO CELL THERAPIES

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The use of pluripotent cells for the correction of cellular defects provides an exciting new approach to medical therapy. Its potential is broad and may provide therapies for diseases that are currently intractable. Embryonic stem cells (ESC) are of particular interest because of their complete pluripotency.

The creation of large banks of ‘excess' human embryo's generated by current practices within the IVF industry provides a rich resource for generation of ESC lines. The utility of such lineages requires their expansion to colonies of sufficient size to allow effective treatment of many recipients. This capacity for essentially infinite expansion is one of the great assets of ESC, yet may create a serious genetic risk.

Several decades of experience with culture of somatic cells in vitro show colony expansion to be associated with a substantial risk of genotoxicty. Currently there is insufficient understanding of the genetic and epigenetic stability of ESC under these same conditions. Furthermore, the genetic and epigenetic stability of cells sourced from IVF embryos remains an open question.

Many changes to the genetic stability of cells enhance their capacity for growth and survival in vitro, and their propensity for tumourogenicity upon transfer. Given the inherent clonality of the system, genetic/epigenetic errors in even a single cell have the potential to contaminate an entire cell line. The detection of such errors challenges the current limits of technology.

Progress in this field requires the development of an understanding of its potential risks, adoption of strategies that mitigate these risks, and adoption of a regulatory framework that balances these risks with potential therapeutic benefits.

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GROWTH HORMONE RECEPTOR SIGNALLING REGULATES THE PREVALENCE OF ADULT NEURAL STEM CELLS

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Reports of growth hormone (GH) effects on precursor cells during development and the presence of GH binding sites on cells located in the adult subventricular zone (SVZ), a region highly enriched with functional neural stem cells (NSCs), prompted us to investigate GH receptor (GHR) as a putative positive NSC marker. Accordingly, we harvested brain tissue from the adult murine SVZ, and sorted viable cells based on the presence (GHR+ve) or absence (GHR-ve) of GHR - as detected by a monoclonal antibody against the cell surface portion of GHR – using flow cytometry. A small (0.3%), yet distinct population of GHR+ve cells were apparent, a subset of which were shown to be bona fide NSCs. As the majority of sphere forming cells were found in the GHR-ve population, this precluded this antigen as a selective NSC marker. We next investigated the role that GH plays on NSCs by; a) exposing serial passed cells to GH, b) infusing GH directly into the ventricles of adult mice, and c) culturing adult SVZ stem cells derived from GHR-/- mice. Data analysis using the Neural Colony Forming Cell Assay and mathematical modeling revealed that GHR plays a role in symmetric stem cell divisions as evidenced by an increase in the number of stem cells with the in vitro addition of GH and a reduction in their numbers in GHR-/-mice. Consistent with these results, we found that infusion of GH into the lateral ventricles for a period of 7 days resulted in a significant and sustained (up to 120 days post-infusion) increase in the number of endogenous NSCs. Given that the absolute numbers of NSCs decline with age, stimulation of such cells via GH or its receptor may represent a novel means by which to reverse or prevent the deleterious effects of aging.

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HOW TO WRITE A SUCCESSFUL GRANT

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Abstract unavailableThis presentation will focus on key elements of a successful grant application.

Preparation: plan ahead – generate preliminary data; start preparation at least 2-3 months ahead; define aims clearly; leave time for others (experienced, successful researchers, collaborators) to read your proposal; listen to criticism and incorporate ideas / suggestions.

Background: ensure clarity of ideas, hypothesis and aims; ensure that the background is well written, logical and supported by appropriate references; present concepts clearly and use illustrations where appropriate; incorporate strong, relevant preliminary data; market your ideas well; emphasize the importance.

Significance: stress the need for the proposed project and the potential health benefits (be realistic – don't simply say that there is potential to develop new diagnostics and therapies); indicate how the findings will advance knowledge and the potential to publish results in high quality international journals; stress the timeliness of the proposal and the potential for scientific impact.

Novelty: innovation and novelty are critical; demonstrate originality and creativity with respect to concept, approach and methodology.

Approach: hypothesis driven science is important; address mechanism rather than being descriptive; the aims should not be interdependent; ensure a detailed, logical experimental design to test the hypothesis; don't be too ambitious (realistic, feasible experiments are very important); discuss controls, statistics (numbers, power calculations), data interpretation, expected outcomes, alternative strategies; include a model if appropriate.

Feasibility: include appropriate preliminary data to support your concept, demonstrate feasibility and convince the reviewers that you have the ability to conduct the research proposal; identify key collaborations; availability of critical reagents; ensure that the methods are established and feasible; include a timeline.

Track record: track record (relative to opportunity) is very important (choice of co-investigators may be very important in this respect); use the track record statement to highlight achievements and establish your credentials (ability to successfully complete the proposal); also use this to identify any issues (eg maternity leave, relocation) that may have impacted on productivity.

Budget: be realistic and adequately justify all aspects of the budget.

Style: be exciting and clear; keep it simple and not too dense; use sub-headings; make it easy to read; include pictures, figures, cartoons and models as appropriate; don't irritate your reviewers by making the application too dense, too complex and too difficult to follow; remember the importance of clarity, literacy, grammar, spelling, layout – if your writing is sloppy, may be your science is sloppy!

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THE IMPORTANCE OF MENTORS

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Having a good mentor can be a defining influence on careers in research, yet the importance of establishing and maintaining mentoring relationships is not frequently emphasised. Mentors can be immediate supervisors, and can also be peers, colleagues in similar or different areas of research, members of peer-review committees, and more generally members of professional societies such as ESA. There are multiple mentoring models, and many attributes of being a good mentor. There are also key factors to consider when choosing a mentor. Above all, a mentor reminds us that we are not alone in negotiating our common professional journey.

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GETTING PUBLISHED

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Getting your article published can be a rewarding experience. However for many this is a tortuous unpleasant experience. There a few steps that can help you but none are completely fool proof.

Match your expectation with that of the journal. Identify how the journal is managed (Scientific society or publishing house or on line etc), how you think reviewers are chosen (editorial board members, database of authors etc) either directly or via a sub editor. Is the journal publishing material like yours and on your topic. Is it aspiring to increase its impact factor, its readership, change its content, or be more profitable? Does it have a limit on number of pages published. Some journals do not have a high impact but have a high rejection rate. How many articles are original research and how many are reviews? Which country is the journal based in, and where will it source its reviewers from.

Present a well written article that looks good and reads well. Many reviewers get turned off by poor quality articles and tend to rank them lower. Conversely something well written, well presented and with catchy figures usually gets a higher ranking just because of the presentation. Sometimes even a colour fig will help the editor err on the side of acceptance rather than a rejection.

On replying to an editor, never assume the editor will handle the response. Sometimes there can be a new editor. Or they find it too complex to follow and so return it to either the original reviewers or new ones. So be prepared to answer correctly and succinctly and

avoid antagonizing anyone. Surprisingly about >90% of reviewers write private comments to the editor. Some of these do not gel with what they write to the author.

When should you resubmit to another journal. That is easy if its complete rejection, but sometimes you undertake all the work requested, only to find it is still rejected. This is annoying and is weakness of larger journals who do not handle and comment individually on articles. Some of the form letters from journals are not very clear about where your article stands, or the chances of success on resubmission.

The list of do and do nots is endless, but basically the trick is to match your paper with the journal to increase your success rates and enjoyment at having your articles published.

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GNRH AND ALL THE PRESIDENT'S MEN (A CONSPIRACY THEORY)

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GnRH was discovered in 1970 and is the primary driver or the reproductive axis. The discovery significantly advanced the field of neuroendocrinology, providing final proof of the neurohumoral theory of Harris. Over the last 36 years, major milestones have shaped our understanding of how the regulation of secretion of GnRH and its action on gonadotropes.

GnRH cells do not express the major steroid receptors, so identification of the neural elements that relay steroid feedback to the GnRH cells has been a major pre-occupation. The recent discovery of kisspeptin has been a major advance in this regard. Sex steroids act at various levels in the brain to control the system through an arrangement of serial and converging pathways. These ‘conspire' to either extract maximal ‘returns' through positive feedback or ‘collaborate' to effect negative feedback. Other developments have required revision of the model of one neurohormone (GnRH) acting on the gonadotropes. Thus, identification of gonadotropin inhibitory hormone (GnIH) demands a thorough revision of neurohumoral control of reproduction; GnIH is a *bona fide* member of the ‘Board'. Collusion between different neurotransmitters may occur in various ways, apart from providing multiple input to GnRH cells. One possibility is that certain key neurons produce multiple transmitters. Another possibility is reciprocal communication between systems that have multiple functions, such that information can be processed in relation to more than one input and/or output.

At the level of pituitary gonadotrope, GnRH acts to control synthesis and secretion of gonadotropins, but various factors ‘conspire' to modulate action. A recent development has been the revelation that extremely rapid estrogen action is exerted on the gonadotropes to phosphorylate second messengers. This is manifest in the alteration of intracellular free calcium and luteinising hormone secretion. Accordingly, our model of the gonadotrope function requires revision. The rapid effects of estrogen as opposed to the more prolonged actions explain the sequential negative and positive feedback action.

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GHD IN ADULTS AND ACROMEGALY

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GHD in adults

Hypopituitary adults with are known to have reduced life expectancy with a 2-fold higher risk of death for cardiovascular disease compared with healthy controls. GHD has been considered the underlying factor of the increased mortality, although a recent study from UK did not show any relationship between GHD and cardiovascular mortality in a small part of the entire cohort of the patients. Although many other factors, such as excessive glucocorticoids or thyroxin replacement, gonadal steroids under-replacement, can potentially contribute to the increased cardiovascular mortality in hypopituitary patients, the direct effect of GHD is highly reliable. GHD can play negative effects on the cardiovascular function both directly on the heart and endothelium and indirectly via hypercoagulability, abdominal obesity, insulin resistance, high total and LDL-cholesterol and low HDL-cholesterol, atherosclerosis, decreased exercise performance, pulmonary capacity and endothelial function. Besides the cardiovascular risk factors mentioned above, patients with GHD were shown to have increased blood vessel intima–media thickening (IMT), that is well known to represent one of the earliest morphological changes in the arterial wall in the process of atherogenesis. Finally, GHD modifies cardiac size and function. Long-term GH replacement therapy reverses most of these abnormalities, at least partly.

Acromegaly

Today, treatment options for acromegaly include surgery, irradiation and pharmacological suppression of GH levels by means of dopamine agonists (DA), somatostatin-analogues (SSA), either alone or in combination, and GH receptor antagonist. Surgical removal of the pituitary adenoma still remains the first therapeutic option in most cases, although primary pharmacotherapy has been recently proposed. Preliminary studies have clearly demonstrated the effectiveness of the GH-receptor antagonist in suppressing IGF-I levels but data on tumor mass are still scant. SSA are very effective in patients with acromegaly either when given as primary or as adjunctive therapy after unsuccessful surgery. The available formulations of octreotide-LAR and lanreotide-autogel are also very well tolerated by the vast majority of the patients. Unfortunately, one third of the patients do not satisfactorily respond to SSA. This is likely due to the fact that octreotide and lanreotide bind to somatostatin receptor subtype 2 and 5 while not every GH-secreting tumor express high numbers of these two receptor subtypes. Therefore the possibility that these are not the best analogues for treating acromegaly is likely. In the next future, new molecules able to bind other receptors, or to bind somatostatin and dopamine receptors, are expected to increase the therapeutical possibilities of acromegalic patients.

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ENHANCED PREGNANCY RATES FOLLOWING LOW DOSE INSEMINATION WITH SEX-SORTED RAM SPERM

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While modified flow cytometry is the only effective method for selecting sex prior to conception, this technology is constrained by high costs associated with inefficiencies in the sorting process. These limitations may be overcome by using a lower sperm dose and/or more controlled timing of insemination. A previous study reported similar fertility rates following insemination of either 1 or 15 million motile sorted sperm[1]. Therefore, a field trial was conducted with the aim of comparing various insemination times at the aforementioned dose rates. Semen was sorted from 3 Merino rams into high purity X- and Y-chromosome bearing sperm populations. Ovulation was controlled in 732 Merino ewes using PMSG at progesterone pessary removal and GnRH 36h later. Sorted (S) and non-sorted (NS) doses of $1x10^6$ ($_1$) and $15x10^6$ ($_{15}$) motile, frozen-thawed sperm were inseminated laparoscopically at 50, 54, 58, 62 and 66h after pessary withdrawal. Pregnancy was diagnosed by ultrasound at 60-62d. Both $1x10^6$ and $15x10^6$ dose rates achieved similar pregnancy rates when inseminated at 58h (S_1: 19/39, 49%; S_{15}: 17/39, 44%; NS_1: 16/39, 41%; NS_{15}: 19/39, 49%). The S_1 treatment produced pregnancy rates that did not differ between insemination times, whereas those obtained using the NS_1 treatment were lower at all time points other than 58h (50h: 1/18, 6%; 54h: 1/39, 3%; 58h: 16/39, 41%; 62h: 8/39, 21%; 66h: 3/18, 17%). Overall, sorted sperm displayed higher pregnancy rates compared with non-sorted sperm ($P = 0.011$). These results indicate that following low dose insemination, sorted sperm have a longer viable lifespan within the female reproductive tract compared with non-sorted sperm. We hypothesise that the sorting process selects a homogeneous, fertile sub-population of sperm, removing weak, damaged and morphologically abnormal cells. These results affirm the position of sex-sorting technology as an important reproductive tool for the future breeding management of sheep.

(1) de Graaf et al. (2007) Reproduction in Domestic Animals (In Press).

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ACTIVIN SIGNALLING MODULATORS IN NORMAL, HORMONE-TREATED, AND NEOPLASTIC HUMAN TESTIS.

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We recently identified Sertoli cells, spermatogonia and spermatocytes as activin signalling targets in human testis, based on the selective production of three activin receptor subunits in these cells. We also observed upregulation of the fourth activin receptor subunit as pre-malignant carcinoma in situ (CIS) cells progress to form malignant germ cell seminomas. Given the importance of regulated activin signalling in testis development and function, the presence of activin signalling transducers (phosphorylated Smad2/3) and antagonists (inhibin α, betaglycan, Smad6) was determined in Bouins fixed, paraffin embedded adult human testis sections using immunohistochemistry. Additional samples examined were from testicular carcinoma patients and from normal men subjected to hormonal suppression with androgen-based contraceptives. In normal and gonadotropin-deprived testes, all antigens were readily detected in Sertoli cells. In addition, Smad6 and phosphorylated Smad2/3 were observed within spermatogonia and spermatocytes, while betaglycan was detected in round spermatids. A strong Smad6 signal was evident in all CIS and seminoma cell nuclei, which exhibited phosphorylated Smad2/3. While inhibin α and betaglycan were not detected in CIS cells, seminomas showed heterogeneous inhibin α and betaglycan signals; most samples (7 out of 9) showed low to undetectable levels of both, while few (2 out of 9) demonstrated strikingly strong inhibin α and betaglycan staining. This differential expression of betaglycan in testicular carcinomas was verified by *in situ* hybridisation, with *betaglycan* transcripts detected in seminoma cells. Detection of phosphorylated Smad2/3 in all analysed seminoma samples indicates activin signalling persists in tumor cells and the detection of inhibin α and betaglycan in some tumors indicates these factors may alter activin bioavailability in a subset of cancers.

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SPECIFIC NUCLEAR TARGETING OF CHROMATIN REMODELLING FACTOR, CDYL BY IMPORTIN A2 IS CRITICAL FOR ITS ROLE IN HISTONE H4 HYPERACETYLATION DURING SPERMATOGENESIS

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Spermatogenesis is a unique and ordered process governed by changes at the level of the nucleus in gene expression. This may partially be controlled through the regulation of the nuclear transport machinery, including importins (IMPs). We have previously demonstrated that several IMPs (α1, α2, α3, α4, α6, β1 and β3) are developmentally regulated during mouse spermatogenesis, suggesting that individual IMPs have specific regulatory roles in spermatogenesis by transporting particular cargo(es) at distinct stages of differentiation. Identification of IMP cargoes in the testis should help describe the potential developmental switches critical

to the spermatogenic process. We performed a yeast two-hybrid screen using an adult mouse testis library and full length IMPα2 as bait, identifying Chromodomain Y chromosome-like, Cdyl as an interactor. Mouse Cdyl has been reported by others to be predominantly expressed during spermiogenesis and to participate in the hyperacetylation of histone H4, which is understood to facilitate protamine replacement of histones during spermiogenesis. Cdyl-IMPα2 interaction was verified by co-immunoprecipation and cotransfection approaches, while immunohistochemical staining of adult mouse testis sections indicated their expression in elongating spermatids. Importantly, increasing Cdyl nuclear accumulation in HeLa cells by overexpressing IMPα2 can increase histone H4 hyperacetylation; prevention of Cdyl nuclear localisation markedly reduced histone H4 hyperacetylation. More importantly, IMPα2 (compared to other IMPαs) was the most efficient in effecting both Cdyl nuclear import and histone H4 hyperacetylation. Our current hypothesis is that IMPα2 mediates nuclear targeting of Cdyl to facilitate its role in histone H4 hyperacetylation during protamine-histone exchange in the final stage of spermatogenesis.

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GONADOTROPINS REGULATE GERM CELL SURVIVAL NOT PROLIFERATION, IN NORMAL ADULT MEN

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Gonadotropins support spermatogenesis via poorly understood mechanisms. We aimed to identify the role of FSH and LH in regulating germ cell apoptosis and proliferation in normal fertile men. Testicular tissues were obtained after gonadotropin suppression induced by testosterone (200mg i.m. weekly) alone or combined with depot medroxyprogesterone acetate (300mg i.m. once) for 2 or 6 weeks (n=5 or 10 men/ group) and an untreated group served as controls. Apoptosis and proliferation were identified by TUNEL and PCNA labelling methods, respectively. Intrinsic and extrinsic apoptotic pathways were identified by immunohistochemistry using the pathway-specific proteins: activated caspase (aCaspase) 9 and 8 and quantified using stereological techniques. By 2 and 6 weeks, the proportion of TUNEL-labelled spermatogonia increased to 354% and 268% of controls ($p<0.001$), respectively, with increased caspase 9 (223% and 166% of controls ($p<0.001$)) but no increase in caspase 8 immunoreactivities. At 6 weeks, the proportions of TUNEL-labelled spermatocytes and round spermatids tended to increase (303% and 180% control, NS), as did caspase 9 (199% and 147% of control, NS) and caspase 8 immunoreactivities (286 and 243% of control, $p=0.4$ and $p=0.06$), respectively. The proportion of TUNEL-labelled elongating/elongated spermatids tended to increase (144% and 138% of control, NS respectively) at 2 and 6 weeks with no change in either caspase immunoreactivities. The number of PCNA-labelled cells did not differ between treated and control men. We demonstrated that gonadotropins act as spermatogonia survival factors via the regulation of intrinsic apoptotic pathway while having no effect of cellular proliferation in normal men.

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"JOEY" – A NOVEL MODEL OF MALE SPERMATOGENIC FAILURE GENERATED USING ENU-MUTAGENESIS

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In order to identify genes critical to fertility, we have screened libraries of N-ethyl nitrosourea (ENU) mutagenised mice containing a series of random mutations for infertility phenotypes and have identified a novel model which we have designated "Joey". Female Joey mice have normal fertility, but male Joey mice are azoospermic and sterile. Both male and female mice appear otherwise healthy. An analysis of t estis histology revealed that spermatogenesis proceeds normally up to step 8 of spermiogenesis after which few spermatids are seen. Spermatids are lost by sloughing and can be seen in the process of degenerating in the epididymis. Germ cell loss occurs in a time frame concordant with the initiation of sperm tail development and formation of the characteristic head shape. Most promising however, is that this period is also critically sensitive to androgen signalling and is time that the unique cell junction type, the ectoplasmic specialization, forms between spermatids and the supporting Sertoli cells. Testis weight in adult affected mice is 50% lower than that of wild type litter mates.

In order to identify the causal mutation, linkage analysis was performed using an array based method and a 3 Mb region on chromosome 9 mapped as the causal region. The region contains 89 genes, a number of which have been selected for direct DNA sequencing based on expression location and timing. The identity of the causal mutation will be confirmed using a Bacterial Artificial Chromosome (BAC) transgenic rescue approach .

The Joey mouse model of male infertility described herein is unique and affects a relatively poorly understood aspect of the transition from a round spermatid into a highly polarised sperm. These mice represent a valuable tool both for our understanding of the mechanism of spermatogenesis and the development of novel male gamete based contraceptives.

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IDENTIFICATION OF CHAPERONE ASSOCIATED PROTEINS POTENTIALLY INVOLVED IN GAMETE RECOGNITION AND INTERACTION.

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Mammalian testicular spermatozoa, are incapable of fertilisation. Before fertilisation can occur, spermatozoa must undergo both epididymal maturation in the male reproductive tract and capacitation in the female tract. Only sperm that have traversed the epididymis attain the functional endpoints of capacitation, the ability to acrosome react and fertilise an egg. Capacitation is correlated with an increase in the level of tyrosine phosphorylation of a number of proteins, several of which become exposed on the cell surface.

Previous analysis of the surface phosphoproteome of capacitated sperm demonstrated that the molecular chaperone heat shock protein 60 (Hsp60) is exposed on the plasma membrane overlying the acrosome, an ideal position for interaction with the zona pellucida. Although Hsp60 is not directly involved in zona binding, it has been proposed that during capacitation, intracellular tyrosine phosphorylation activates Hsp60 inside the cell, orchestrating the assembly of a zona binding complex and its subsequent exposure on the outside of sperm. To investigate the role of Hsp60 in fertilization, a proteomics-based approach was employed to identify chaperone associated proteins in capacitated sperm. Hsp60 was immunoprecipitated from capacitated sperm and associated proteins identified by liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis. Protein interactions were confirmed by reciprocal immunoprecipitation followed by Western blot analysis. To this end we have identified a number of proteins including an aldose reductase-related protein and the proacrosin binding protein. Both proteins co-localise with Hsp60 to the acrosomal region. However, this expression pattern is lost once sperm have undergone calcium ionophore A23187 induced acrosome reaction, as would be expected of molecules potentially involved in sperm-egg interactions.

Based on these data we hypothesize that during epididymal transit, proteins important for fertilisation are deposited on sperm. During capacitation these proteins are assembled into functional protein complexes by chaperones including Hsp60, and chaperoned to sites including the cell surface where they affect the functional competence of sperm.

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EXPOSURE TO A HIGH PHYTOESTROGEN DIET FROM CONCEPTION REDUCES SERTOLI CELL NUMBER IN THE 18 DAY OLD RAT.

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Environmental oestrogens have been implicated in the decline in human sperm counts. We have shown that exposure to a high phytoestrogen diet from conception reduces the number of Sertoli cells and spermatogonia in the adult rat. This study investigates when this reduction in cell number occurs. Male rats and their mothers were fed a high phytoestrogen (HP, 456?g/g isoflavones) or phytoestrogen-free (PF) diet from conception to death. Groups (n=10) of HP and PF rats were killed at 5 and 18 days post-partum. The testes were removed, embedded, cut into 30?m sections, and stained with haematoxylin and eosin. The number of spermatogonia, Sertoli and Leydig cells per testis were estimated using the optical disector method, combined with the Cavalieri method. To compare cell proliferation at 18 days post-partum, PF and HP treated rats (n = 10) were injected with bromodeoxyuridine (BrdU) two hours prior to death. Immuno-histochemistry was used to identify BrdU labelled cells and immuno-positive cells counted using the physical disector method. At 5 days postpartum, the number of spermatogonia per testes were increased in HP rats ($P<0.02$). No difference in Sertoli or Leydig cell number was observed. At 18 days, the number of Sertoli cells per testis was significantly reduced ($P = 0.04$) in HP rats. No significant differences were observed in the number of Leydig cells, spermatogonia or BrdU immuno-positive cells per testis between groups. This study indicates that following exposure to a high phytoestrogen diet from conception the number of Sertoli cells per testis is reduced at 18, but not 5, days postpartum. While early exposure to a HP diet may increase the number of spermatogonia at 5 days postpartum, this increase is not sustained when animals are maintained on a HP diet.

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STAGE-DEPENDENCY OF TESTICULAR TIGHT JUNCTION LOCALISATION AND EXPRESSION.

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Sertoli cell tight junctions (TJ) form the blood-testis barrier, are regulated by gonadotrophins[1], and are indispensable to spermatogenesis[2]. Recent studies have achieved contraceptive efficacy by targeting the blood-testis barrier[3], although little is known about TJ dynamics in normal spermatogenesis.

This study aimed to associate the localisation and expression of TJ proteins at various stages of germ cell development with the functionality of the blood-testis barrier. We hypothesise that these characteristics of TJ proteins vary over the cycle of the seminiferous epithelium. Adult Djungarian hamster testes were excised, injected with a biotin tracer[4], Bouin's fixed and paraffin embedded before processing for confocal microscopy. An anti-claudin-11 (CL11) antibody was used to detect TJ, PNA to label spermatid acrosomes and facilitate staging, and streptavidin-Alexa 488 to localize the qualitative biotin tracer for inference of blood-

testis barrier functionality. Stages were grouped I-III, IV-VI, VII-VIII, IX-X and XI-XII and localisation was classified as basal, intermediate or apical relative to germ cells residing on the basement membrane. In stages I-III, localization of CL11 was predominantly at the basement membrane (72% basal, 9% intermediate, 0% apical). Localisation was intermediate in stages IV-VI (22% basal, 63% intermediate and 4% apical) and predominantly apical during stages VII-VIII (0% basal, 29% intermediate and 71% apical). Localisation returned to a more basal phenotype at stages IX-XII. The frequency of CL11 expression was highest at stages VII-VIII (91%) and IX-X (82%) and lowest in stages I-III (42%) and XI-XII (48%). At all stages, biotin tracer permeated the seminiferous epithelium only as far as CL-11 expression.

These results clearly demonstrate a stage-dependant expression of TJ protein CL11 over the cycle of the seminiferous epithelium. In addition, the localization of CL11 dictates the extent to which biotin is able to permeate the epithelium, and that this functionality also exhibits stage-dependency. The regulation of TJ dynamics in this model after hormonal suppression and replacement is currently under investigation.

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BOVINE SERTOLI CELLS SURVIVE AND FORM TUBULAR STRUCTURE IN AND OUT OF MOUSE TESTIS

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Despite many attempts to achieve transplantation of domestic animal germ cells into the testes of mice or into a host of the same breed, success is rarely achieved. Our group has recently published results indicating that bovine testis donor cells can survive in bovine host testes for over half a year. For donor cell identification, a uniform living cell dye (PKH26) was taken up by donor cells before transplantation; however, unequivocal identification of donor cell types in the host testes was not achieved.

In this study, development of immature bovine testicular tissue was assessed after transplantation into nude mice. Testis tissue was either (a) grafted under the skin, (b) enzymatically digested, then purified cells transplanted into testis tubules, or (c) grafted as subcutaneous cell aggregates. Tissues were collected 2-3 months after surgery for immunohistochemical analysis. We employed several antibodies that we have ascertained are capable of discriminating between bovine and mouse germ cells and Sertoli cells. Our results showed: 1) immature bovine Sertoli cells can survive in mouse seminiferous tubules and in both subcutaneous tissue and cell grafts, forming and maintaining a seminiferous tubular structure; 2) very few bovine germ cells survived in mouse testis, and those surviving germ cells (spermatogonia) did not differentiate, even embedded within bovine Sertoli cells present in the mouse testis; 3) very few germ cells survived in the subcutaneous grafts or aggregates.

These results indicate that bovine germ cells do not survive or differentiate well in mouse testes not only due to the local spermatogenic environment, but also to the systemic environment provided by the mouse. However, this species-specific spermatogenic environment does not appear to prevent cross-species Sertoli cell survival and development .

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THE IMPACT OF ESTROGENIC COMPOUNDS ON DNA INTEGRITY IN THE MALE GERM LINE

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DNA damage in the male germ line is associated with poor fertilization rates following IVF, defective preimplantation embryonic development, and high rates of miscarriage and morbidity in the offspring, including childhood cancer. This damage is poorly characterized, but is known to involve oxidative base damage, endonuclease-mediated cleavage and the formation of adducts with xenobiotics as well as reactive metabolites. Estrogen metabolites such as 2, and 4-hydroxyestradiol, collectively known as catechol estrogens, are inherently more reactive than estrogen itself and are also more redox active compounds. Recent data suggests that similar estrogenic compounds can directly induce DNA damage in human spermatozoa via the stimulation of intracellular redox activity. We have undertaken a detailed analysis of this relationship using a range of techniques validated for use in these highly specialized cells. DNA damage (Comet and TUNEL assays), the redox state of the cell (dihydroethidium/Sytox Green assay) as well as standard sperm parameters (motility and vitality) were measured after exposure to a range of estrogens. After exposure to catechol estrogens an unusual suppression of damage was observed by the Comet assay. The negative control showed a familiar damage value of 46% while the 2-hydroxyestradiol (25 m M) treated cells yielded a value of 3% ($P < 0.001$). This suggests that the chromatin is being physically bound together. Increases in reactive oxygen species were also observed. We concluded, that catechol estrogens while contributing to oxidative stress, also tightly cross-linked the chromatin of spermatozoa *in vitro*. The cross-linking was also supported by electrospray ionisation mass spectrometry data. These types of lesions have important implications for the viability of human sperm cells. Severe chromatin cross-linking can potentially prevent decondensation of the affected sperm nucleus in the oocyte and therefore, prevent fertilisation. These findings raise fundamental questions about the generation and metabolism of

catechol estrogens in the testes and epididymis, and their role in creating the unusually high levels of sperm DNA damage that characterises our species.

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VITAMIN D AS AN ANALGESIA FOR PATIENTS WITH TYPE 2 DIABETES AND NON-SPECIFIC MUSCULOSKELETAL PAIN - A PROSPECTIVE OBSERVATIONAL STUDY.

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Aim: It is common in clinical practice to encounter the diagnostic challenge of non-specific musculoskeletal pain in patients with type 2 diabetes. While the association between hypovitaminosis D and myalgia is well known, the impact of vitamin D insufficiency on pain in diabetic patients has not been evaluated. Our study aims to study the impact of vitamin D repletion on musculosketetal pain in vitamin D insufficient patients with type 2 diabetes. Method: 51 patients with type 2 diabetes who presented with diffuse myalgia/arthralgia were included. Severity of pain was evaluated by the McGill pain questionnaire and 5cm visual analogue self-report scales (0=no pain; 1=mild; 2=discomforting; 3=distressing; 4=horrible; 5=excruciating). Serum 25-hydroxy vitamin D (25D) concentration and ionized parathyroid hormone (iPTH) concentration were measured. Patients with vitamin D insufficiency (serum 25D concentration <60 nmol/L) were supplemented with vitamin D3, and were reviewed in 3 months with repeat measurement of biochemistry and re-evaluation of pain. Results: All patients were vitamin D insufficient with mean serum 25D concentration of 44 nmol/L. Pain scores on the visual analogue scale (r= -0.32) and the McGill questionnaire (r= -0.43) correlated negatively with serum 25D concentration but not serum iPTH concentration. Repletion of vitamin D with supplement resulted in significant reduction in pain scores on both the visual pain analogue scale and McGill pain questionnaire at -48.5% and -39.4% respectively. Conclusion: Mean serum 25D concentration in our patients was higher than osteomalacic patients reported in the literature (44 nmol/L vs <30 nmol/L). Vitamin D insufficiency may be additive to hyperglycaemia in impairing nociceptor function in diabetic patients, resulting in pain at a threshold of serum 25D concentration higher than the non-diabetic population. Early recognition of the association of non-specific musculoskeletal pain with vitamin D insufficiency in the diabetic population reduces patients' anxiety, unnecessary referrals, and vitamin D repletion may reduce symptoms.

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HEALTHIER LIFESTYLE PREDICTS HIGHER SUBSEQUENT SERUM TESTOSTERONE AND SEX HORMONE BINDING GLOBULIN CONCENTRATIONS IN OLDER MEN. THE HEALTH IN MEN STUDY.

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Introduction and research aims: Testosterone concentrations decline during male ageing but it is unclear whether healthy lifestyle preserves circulating testosterone. We evaluated the relationship between lifestyle score with subsequent sex hormone status to test the hypothesis that healthier lifestyle would result in higher testosterone levels in older men.

Methods: Longitudinal evaluation of 3,453 community-dwelling men aged 65-83 years at baseline was performed. Lifestyle score, a tally of 8 prudent health-related behaviours, was determined during 1996-1999. In 2001-2004, early morning sera were collected for assays of total testosterone, sex hormone binding globulin (SHBG) and luteinizing hormone (LH). Free testosterone was calculated using the Vermeulen method.

Results: Time between collection of lifestyle data and blood sampling was 5.7±0.9 years (range 3.3-8.2 years). Lifestyle score correlated with subsequent serum total testosterone (Spearman's rho=0.06, p<0.0001) and SHBG (r=0.07, p<0.0001), but not with free testosterone (r=0.03, p=0.08) or LH (r=-0.03, p=0.12). Total testosterone and SHBG differed significantly across lifestyle score (p=0.007 and p<0.0001, respectively) and increased with increasing lifestyle score (p<0.0001 for both). In multivariate analysis adjusting for age and physical co-morbidity, low lifestyle score predicted total testosterone and SHBG in the lowest quartile of values (lowest lifestyle score ≤1; odds ratio: 2.64, 95% CI: 1.28-5.44 for total testosterone <11.7 nmol/L, and OR: 3.69, 95% CI 1.80-7.57 for SHBG <31.4 nmol/L).

Conclusions: In men >65 years old, higher lifestyle score reflecting greater engagement in healthy behaviours predicts higher total testosterone and SHBG after a 5.7 year interval. Further investigation is warranted to clarify the interaction between behaviour, hormone status and health outcomes during male ageing, particularly whether promoting healthy lifestyle behaviours might ameliorate the age-related decline in testosterone concentration and improve health.

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GLUCOCORTICOID-INDUCED PROTEIN WASTING: PROTEIN METABOLIC EVALUATION OF THE THERAPEUTIC POTENTIAL OF GROWTH HORMONE AND DEHYDROEPIANDROSTERONE

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Therapeutic use of glucocorticoids (GC) causes substantial morbidity from protein wasting. No therapies are available to prevent GC-induced protein loss. We investigated whether growth hormone (GH) and dehydroepiandrosterone (DHEA) can induce protein anabolism in chronic GC users. In an open, stepwise, dose-finding study (Study 1), 6 subjects (3 women, age=69±4yrs) on long-term (>6 months) GCs (prednisone dose=8.3±0.8mg/d) were studied before and after two sequential GH doses (0.8mg/d and 1.6mg/d for two weeks each), approximating 2 and 4 times daily production rates. Based on these results, 10 women (age=71±3yrs) on long-term GCs (prednisone dose=5.4±0.5mg/d) were studied in an open, randomised crossover study (Study 2) at baseline and after two weeks GH 0.8mg/d, DHEA 50mg/d and combined GH and DHEA treatment. Changes in whole body protein metabolism were assessed using a 3-h primed constant infusion of 1-[13C] leucine, from which rates of leucine appearance (LRa, an index of protein breakdown), leucine oxidation (Lox, index of protein oxidation) and leucine incorporation into protein (LIP, index of protein synthesis) were estimated. In Study 1, GH reduced Lox (p=0.03) and increased LIP (p=0.02) in a dose-dependent manner, while LRa did not change significantly. In Study 2, GH significantly increased LIP (Δ+7.3±2.6μmol/min, p=0.02) and reduced Lox by 10% (Δ-1.7±1.3μmol/min, p=0.17), although the change was not statistically significant. DHEA did not significantly affect LRa, Lox and LIP. Combination treatment increased LRa (Δ+6.1±2.8μmol/min, p=0.048) and LIP (Δ+8.4±2.7μmol/min, p=0.007) and reduced Lox (Δ-2.3±1.1μmol/min, p=0.04). Fasting glucose increased by ~0.4mmol/L with GH and combination treatment. In summary, modest supraphysiological GH doses reduced protein oxidation and increased synthesis in subjects on long-term GCs in a dose-dependent manner. DHEA alone exerted minimal effect, but augmented the anabolic action of GH. We conclude that GH and DHEA have therapeutic potential to prevent protein loss induced by GCs. (Supported by NHMRC and Pfizer Australia)

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PILOT CLINICAL TRIAL WITH HUMAN ISLETS IN BARIUM ALGINATE MICROCAPSULES

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ß cells placed in microcapsules of barium alginate normalize blood glucose levels (BGL) of allografted diabetic mice. Encapsulated human islets also normalize BGL of mice. Our aim was to examine the safety and initial efficacy in transplanting encapsulated human islets in people with type 1 diabetes, who were C-peptide negative. Islets were isolated from donor cadaveric pancreases and encapsulated in barium alginate after 2 d culture. They were injected into the peritoneal cavity of 3 C-peptide negative ABO matched recipients with type 1 diabetes 1-3 d later. A total of 6 preparations were transplanted (4 in one recipient) with 173,000 ± 17,000 IEQ per preparation. No immunosuppression was used.

Insulin requirements decreased transiently for the first few days after transplantation, during which time C-peptide was measurable. Values peaked on day 1, and were detectable for 1 week, and in 1 recipient at low levels for 3 months. There were no adverse effects. Islet cell antibodies to GAD but not ICA512 were first detected in 1 of the 3 recipients 1 month post transplantation, and these were still detectable 3 months later. Cytotoxic antibodies to antigens of the grafted islets were detected in 2 of the 3 recipients by 3 weeks post-transplant, and persisted in declining titres for at least 11 months. Analysis of culture medium conditioned by islets prior to transplantation showed that levels of the chemokine MCP-1, but not MIP-1α, MIP-1β, RANTES, eotaxin or IP10, increased after encapsulation - 30.8 ± 5.1 from 13.8 ± 3.9 ng/mL/d. Level of cytokines diminished after encapsulation.

Human islets encapsulated in barium alginate function, although not ideally, when allografted into C-peptide negative recipients with type 1 diabetes. A reason for this is cytokine-induced toxicity, exacerbated by elevated levels of MCP-1. Shedding of antigens from the encapsulated islets after transplantation is likely to be responsible for the recurrence of islet cell antibodies and the production of cytotoxic antibodies. Strategies need to be introduced to block the adverse effect of chemokines/cytokines on the grafted islets.

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THE USE OF ANTI-MULLERIAN HORMONE IN PREDICTING MENSTRUAL RESPONSE FOLLOWING WEIGHT LOSS IN OVERWEIGHT WOMEN WITH POLYCYSTIC OVARY SYNDROME

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Background: Polycystic ovary syndrome (PCOS) is associated with reproductive and metabolic abnormalities, specifically menstrual dysfunction and anovulation in conjunction with elevated pre-antral follicle number and arrested follicular maturation. Although anti-mullerian hormone (AMH), an inhibitor of follicle recruitment and maturation, is increased in women with PCOS, the usefulness of circulating AMH levels as a clinical predictor of menstrual response to weight loss in PCOS is not known.

Methods: Overweight women with PCOS (n=26, age 32.9 ± 1.1 years, weight 98.9 ± 4.1 kg, BMI 36.1 ± 1.4 kg/m^2, mean SD) followed an 8 week weight loss and 6 month weight maintenance program.

Results: Net reductions in weight (4.6 ± 1.0 kg), waist circumference (6.0 ± 1.2 cm), testosterone (0.3 ± 0.1 nmol/L), fasting insulin (3.7 ± 1.7 mU/L) and the homeostasis model assessment of insulin sensitivity (0.7 ± 0.3) occurred for all subjects over the entire study duration. 15/26 (57.7%) subjects responded to the intervention with improvements in menstrual cyclicity (responders). Compared to non-responders, responders had lower AMH levels at baseline (23.6 ± 3.1 versus 37.9 ± 5.4 pmol/L, P=0.021). Only responders had reductions in fasting insulin (6.1 ± 1.6 mU/L, P=0.001) and HOMA (1.3 ± 0.3, P=0.002) with acute weight loss (week 0-8). Baseline AMH was most strongly predicted by baseline ghrelin, free testosterone and insulin (r2=0.528 p=0.002).

Conclusion: Overweight women with PCOS who respond to weight loss with menstrual improvements have significantly reduced AMH and demonstrate improvements in surrogate measures of insulin resistance with weight loss. Pre-treatment AMH is a potential clinical predictor of menstrual improvements with weight loss in PCOS.

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A CASE OF DOPAMINE BETA HYDROXYLASE DEFICIENCY CAUSING SEVERE AUTONOMIC NEUROPATHY AND RESPONDING TO L-DOPS TREATMENT

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Dopamine beta-hydroxylase (DBH) catalyses the conversion of dopamine to noradrenaline. The enzyme is located within the secretory vesicles of noradrenaline producing neurons, sympathetic ganglia and in the cells of the adrenal medulla. It is secreted with noradrenaline by nerve terminals and its plasma activity can be measured.

We describe a case of DBH deficiency in a woman aged 52 years. She gave a history of severe orthostatic hypotension, first documented at the age of 14 years. She experienced recurrent postural syncope. Blood pressure was 80/40 supine, and was unrecordable on standing. Autonomic testing revealed minimal increase in heart rate with upright posture and an abnormal response to Valsalva manoeuvre. There was minimal improvement in symptoms with elastic compression stockings, caffeine, fludrocortisone, ephedrine, dihydroergotamine or octreotide.

Urinary adrenaline was undetectable, <0.02 umol/24h (0.02-0.10). Urinary noradrenaline was low, 0.03 umol/24h (0.20-0.44). Plasma adrenaline was 0.3nmol/L (<1.5) and plasma noradrenaline was 0.8 nmol/L (<3.5), with a plasma dopamine level approximately 8 times normal at 1950 pmol/L (<250). A diagnosis of DBH deficiency was made. Treatment was commenced with oral L-threo-3,4-dihydroxyphenylserine (L-DOPS, Sumitomo, Japan), a synthetic amino acid that is decarboxylated by dopamine decarboxylase to noradrenaline, thereby bypassing the metabolic block. This treatment has resulted in a marked improvement in symptoms with the patient now able to stand, run and record a normal upright blood pressure.

Fourteen cases of congenital DBH deficiency have been reported in the literature. Inheritance is autosomal recessive, with a variety of *DBH* gene mutations identified. Symptoms usually worsen progressively during late adolescence, with severe orthostatic hypotension, eyelid ptosis and inability to exercise by early adulthood. L-DOPS restores plasma noradrenaline to normal and reverses the orthostatic intolerance. This case highlights a rare but severe form of autonomic neuropathy, and the successful use of precursor therapy to bypass the enzymatic deficiency.

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EXPERIENCE WITH LONG ACTING INTRAMUSCULAR TESTOSTERONE UNDECANOATE

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In 2004 we established an Androgen Replacement Clinic to initiate and supervise the treatment of men with hypoandrogenism. Initially the main function of the clinic was to provide access to subcutaneous testosterone implants however since February 2006 a long acting form of intramuscular testosterone undecanoate, Reandron®, has been available. We describe the patient experience of men receiving Reandron® according to a schedule of 1000mg IM administered at Weeks 0, 6, 18 and 30. Men attending for their Week 30 (4^{th}) injection had a pre-treatment serum testosterone measured (during Week 29), and were asked about their treatment experiences and preferences using a 5-point rating scale. In total 92 men aged 18-75 years have attended the clinic. 63 men (60% primary hypogonadism) have received Reandron® with 48 having ³ 4 doses; of these 41 men were assessed. The treatment modality immediately prior to Reandron® was implants (22), transdermal gel (12), Sustanon® (6) or nil (1). Pre-Week 30 testosterone levels were ≤10nM (n=3), 10.1-15nM (n=12), 15.1-20nM (n=13), 20.1-25nM (n=5) and > 25nM (n=3) with no data in 5; there was no association with the previous mode of therapy. Reandron® was rated as unsatisfactory by 1 man, acceptable by 3, satisfactory by 13 and highly satisfactory by 24. Those men who rated the treatment as satisfactory or highly satisfactory noted that it was ‘convenient', ‘effective' and resulted in ‘even testosterone levels'. We conclude that long acting intramuscular testosterone undecanoate provides an effective, convenient and acceptable form of androgen replacement. The variability in nadir serum testosterone levels prior to the Week 30 (4^{th}) injection suggests that clinicians should assess optimum dose intervals on an individual patient basis.

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OVARIAN STEROIDS REGULATE SOCS EXPRESSION IN THE HYPOTHALAMUS: CROSS-TALK BETWEEN STEROID AND CYTOKINE SIGNAL TRANSDUCTION IN THE BRAIN.

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Prolactin stimulates hypothalamic dopamine neurons, mediated by STAT5b. During late pregnancy, however, these neurons cease to respond to prolactin. Suppressors of cytokine signaling (SOCS) proteins are known to inhibit STAT-mediated signalling, and SOCS mRNA is specifically elevated in the hypothalamus during late pregnancy. The are marked changes in levels of ovarian steroid hormones, estrogen (E2) and progesterone (P), during this period. This study aimed to determine whether changing levels of ovarian steroid hormones might alter SOCS expression in the hypothalamus. Adult rats were ovariectomised (OVX) and treated with either blank implants, E2 implants, or E2+P implants. To eliminate possible effects of endogenous prolactin, all animals received 4 subcutaneous injections of the dopamine receptor agonist bromocriptine (200 µg) starting at 0900 h on day 7 of the experiment at 8 hourly intervals. At 0900 h on day 8, animals were injected with ovine prolactin (300 µg sc), or saline vehicle, and killed 2 hours later. Brains were collected for microdissection of the arcuate nucleus and levels of mRNA for SOCS-1, -3 and CIS determined by quantitative RT-PCR. Compared to OVX controls, mRNA for all three SOCS proteins examined was significantly increased following E2 exposure, even though endogenous prolactin was suppressed. Prolonged P treatment completely reversed this effect. Prolactin treatment induced an increase in levels of SOCS-1, -3 and CIS mRNA within the arcuate nucleus compared to untreated OVX controls. For SOCS-1 and CIS, the prolactin-induced increase in SOCS mRNA expression was at least partially prevented following either prolonged E2 or E2+P treatment. In contrast, prolactin-induced SOCS-3 mRNA expression did not change following either steroid hormone treatment. Results demonstrate that changes in E2 and P can alter SOCS expression in response to prolactin, and are consistent with the hypothesis that ovarian hormone changes during late pregnancy mediate changes in SOCS mRNA expression, and thus may contribute to the insensitivity of hypothalamic dopaminergic neurons to prolactin at this time.

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REPRODUCTIVE DYSFUNCTION IN FEMALE ANDROGEN RECEPTOR NULL MICE ($AR^{-/-}$) IS DUE TO EXTRA-OVARIAN DEFECTS IN HYPOTHALAMIC-PITUITARY REGULATION RATHER THAN INTRINSIC OVARIAN DISORDERS

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Recent studies of homozygous $AR^{-/-}$ females, generated using Cre/LoxP recombination, have shown that AR plays a vital role in female reproduction. An in-frame deletion of exon 3, encoding the second zinc finger essential for DNA-binding, created $AR^{-/-}$ females which exhibit dysfunctional late follicle development, ovulation and fertility. Ovulatory dysfunction was identified by decreased numbers of corpora lutea and naturally ovulated oocytes recovered from oviducts after mating. However, reduced ovulation rate was overcome by gonadotrophin hyperstimulation, suggesting an extra-ovarian defect in hypothalamic-pituitary regulation of ovulation. The present study aimed to determine whether the reduced fertility is intrinsic to the ovary or reflects a disruption in extra-ovarian hypothalamic-pituitary regulatory mechanisms. We analysed the timing of sexual maturation (vaginal opening), and following reciprocal ovarian transplantations between $AR^{+/+}$ and $AR^{-/-}$ mice, regularity of estrus cycles and fertility. There was no difference in the timing of onset of vaginal opening. Intact $AR^{-/-}$ females had disrupted estrus cycles (20% did not to cycle) with fewer cycles in 2 weeks (cycles $AR^{-/-}$: 0.8 +/- 0.37; $AR^{+/+}$: 2 +/- 0.00, $P<0.01$) and significantly longer cycles than intact $AR^{+/+}$ females ($AR^{-/-}$: 8.34 +/- 1.64 days; $AR^{+/+}$: 4.6 +/- 0.27days, $P<0.01$). When $AR^{-/-}$ ovaries were transplanted into ovariectomized $AR^{+/+}$ hosts, the $AR^{+/+}$ hosts displayed normal estrous cycles and fertility. In contrast, following transplantation of $AR^{+/+}$ ovaries into ovariectomized $AR^{-/-}$ hosts, the $AR^{-/-}$ hosts displayed abnormal estrous cycles (43% did not cycle), fewer cycles in 2 weeks (cycles $AR^{-/-}$: 0.7 +/- 0.29; $AR^{+/+}$: 1.8 +/- 0.15, $P<0.01$) and reduced fertility (% to produce 1st litter: $AR^{+/+}$ ovaries into $AR^{+/+}$ hosts: 100%; $AR^{+/+}$ ovaries into $AR^{-/-}$ hosts: 43%, $P<0.01$). These results suggest that the subfertility/reproductive dysfunction of $AR^{-/-}$ females is primarily attributable to defective hypothalamic-pituitary regulation rather than intrinsic abnormalities in the ovary. In conclusion the data presented here provides strong direct evidence that central AR-mediated actions are important in maintaining female fertility.

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IS THE SUPPRESSION OF LUTEINISING HORMONE (LH) BY CORTISOL IN GONADECTOMISED EWES AFFECTED BY DIFFERENT LEVELS OF ADIPOSITY?

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Cortisol, a major hormone released during stress suppresses LH secretion although its actions on FSH secretion are less clear. Physiological state can influence the circulating levels of cortisol and we have found that stress causes greater responses in fat ewes than in lean ewes (1). In this study, we tested the hypothesis that an infusion of cortisol (250µg/kg/h) resulting in stress-like levels of cortisol, would reduce plasma LH and FSH levels differently in lean and fat ewes. Ovariectomised ewes were made fat (78.8±2kg) or

lean (36.5±0.3kg) by dietary manipulation and the experiment was conducted as a cross-over design (n=6/group/treatment) with infusion (iv) of saline or cortisol for 30h. In week 1, 3 animals from each group received vehicle or cortisol and then the treatments were crossed over in week 2. Blood samples were taken every 10min for 6h prior to infusion, for 6h after commencement of the infusion and for 6h at the end of infusion. Plasma levels (ng/ml) of leptin, cortisol, LH and FSH were measured by RIA. Leptin concentrations were higher ($P<0.05$) in fat (2.5±0.2) than lean (0.64±0.1) ewes. Fat animals had lower ($P<0.05$) mean levels of LH and FSH than lean ewes. Cortisol infusion increased ($P<0.05$) plasma cortisol levels to a similar extent in fat and lean animals. Mean plasma LH concentrations were reduced ($P<0.05$), in both fat and lean ewes, during the first 6h of the cortisol infusion, but the magnitude of the suppression was greater in lean ewes. No effects of treatment were seen on plasma FSH levels. We conclude that the level of adiposity may affect the ability of cortisol to reduce plasma LH concentrations, but not FSH, in ovariectomised ewes.

(1) Tilbrook A.J. et al 2002 Proc. Int. Congr. Neuroendocr. 5: FC31

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HORMONAL CONTROL OF OVARIAN BLOOD AND LYMPH VASCULAR ANGIOGENESIS: KEY PROCESSES IN FOLLICULOGENESIS AND OVULATION

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Ovulation is a highly regulated process essential for reproductive success, during which oocytes complete meiotic maturation and are released into the fallopian tube. Meanwhile, the follicular structure is remodelled to form the steroidogenic corpus luteum. The blood vascular system is highly critical to this process and provides nutrients, growth factors and hormones, as well as removing waste. We recently identified lymphatic vessels in the ovary and established that lymphatic vasculature develops postnatally, under hormonal control. However, the mechanisms of regulation and function of the ovarian lymphatic system are unknown. This study examined the hormonally-regulated expression of blood and lymphatic vessel markers, as well as angiogenic and lymphangiogenic growth factors and their receptors in ovarian mural granulosa cells, cumulus cells and the stromal/thecal compartment. Each cell type was isolated from groups of eight mice either immature untreated, or following eCG at 4 hourly intervals from 20-44 h (preovulatory folliculogenesis) or at two hourly intervals after hCG (2-16 h; ovulatory follicles). In three independent experiments, we used Real Time PCR, western blot and immunohistochemistry to examine mRNA and protein expression and localisation. Surprisingly, the highly-specific lymphatic endothelial cell marker *Xlkd1* (Lyve1) increased 75±5 fold in granulosa cells following hCG administration. The transcriptional regulator of *Xlkd1*, *Prox1* and the lymphangiogenic growth promoter, *Vegfc* were also increased significantly in granulosa cells following hCG and constitutively present in lymphatic vessels in the stroma. Significant regulation of the blood vascular angiogenic factor, *Vegfa* and the Vegf-receptors *Flt1*, *Kdr* and *Flt4* was also identified in granulosa cells and were abundant and unregulated in blood vessels in the ovarian stroma. This is the first study to identify lymphatic markers in the ovary and also to characterise, in detail, the hormonal regulation and spatial distribution of blood and lymphatic angiogenic regulatory genes in folliculogenesis and ovulation. We propose a multi-faceted role for granulosa cells in promoting tissue remodelling and dynamic neovascularisation during ovulation and corpus luteum formation.

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ANDROGEN RECEPTOR-DEPENDENT STIMULATION OF STEROIDOGENESIS IN A HUMAN GRANULOSA TUMOUR CELL LINE, KGN

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In polycystic ovary syndrome (PCOS), excess androgen is secreted into the follicular environment, which hypothetically disrupts androgen receptor (AR)-mediated signalling within granulosa cells (GC) and contributes to ovarian pathology. The ability to address this hypothesis is hampered by the paucity of appropriate human tissue. While GC collected from women receiving infertility treatment are plentiful, these highly luteinised cells lack AR protein. We previously verified AR expression and characterised androgen responses in a human ovarian granulosa tumour cell line, KGN(1) to define an appropriate model. Androgen enhances steroidogenic activity of KGN cells exposed to follicle stimulating hormone (FSH), similar to animal GC models. In the current study, we investigated whether this activity is mediated via the AR, and examined the effect of increased doses of androgen on steroidogenesis. KGN cells were exposed to combinations of FSH (50-100 mIU/ml), cAMP (1mM), 5-alpha-dihydrotestosterone (DHT; 1-100nM), and two AR antagonists, hydroxyflutamide (OHF; 10-1000nM) and bicalutamide (BIC; 10-1000nM). Oestradiol or progesterone concentration in conditioned media was measured by RIA. A physiological dose of DHT (1nM) significantly increased both oestradiol and progesterone secretion by at least 2-fold over that induced by either FSH or cAMP alone ($p<0.01$). OHF and BIC antagonised this stimulatory effect of DHT on cAMP-stimulated steroid secretion, but only when present at a minimum of 100nM. OHF alone had agonistic effects similar to DHT at a doses of 100nM or 1000nM, but BIC did not. Higher doses of DHT had reduced (10nM) or no (100 nM) ability to enhance FSH- or cAMP-induced steroidogenesis. Our results demonstrate that DHT acts via the AR to enhance steroidogenesis and that OHF acts as a partial agonist in KGN cells. They also support the concept that a hyperandrogenic environment may perturb AR signalling and thereby contribute to the diminishment of steroidogenic output characteristic of GC from polycystic ovaries.

(1) Hickey TE and Norman, RJ. Proc. 46th Annual Meeting of the Endocrine Society of Australia, Melbourne. Abstract # 169, pg 120.

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IN VIVO PRIMING TO PATERNAL ANTIGENS IS CONSISTENT WITH SUCCESSFUL PREGNANCY OUTCOME

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A major factor in pregnancy success is conceptus evasion of immunological attack. The means by which maternal immune tolerance is established and maintained is not clear, however in other tissues the events of initial lymphocyte activation is the key determinant of lymphocyte phenotype and their cytotoxic or tolerogenic effector function. The aim of this study was to evaluate whether activation of T-lymphocytes reactive with paternal antigens either in vitro or in vivo can impact their ability to elicit cytotoxic rejection of the conceptus. Transgenic Act-mOVA male mice that express the model paternal antigen, ovalbumin (OVA), were mated to C57BL/6 (B6) females, to generate conceptuses expressing OVA. Pregnant mice received either naïve or in vitro stimulated cytotoxic OVA-reactive T cells (OT-I) on day 4 of pregnancy. At day 18 of pregnancy in B6 x Act-mOVA matings there was no significant difference in viable pregnancy rate or litter size in mice that received naïve OT-I cells. While pregnancy rate was not reduced in B6 x Act-mOVA mating when cytotoxic OT-I cells were administered, litter size was reduced significantly by 46% ($p < 0.05$). Correspondingly, fetal resorption rate was increased 3.9 fold ($p < 0.05$) in mice receiving cytotoxic cells. The antigen specificity of this effect was confirmed in B6 x B6 matings where pregnancy rates and litter sizes were unchanged after administration of cytotoxic OT-I T cells. Across all 3 groups, there was no difference in fetal or placental weights after administration of naïve or cytotoxic OT-I cells. These data show that paternal-antigen reactive cytotoxic T cells can induce rejection of the conceptus, and that naïve OT-I cells, despite undergoing immunological priming to conceptus antigens, mature into effector phenotypes consistent with immune tolerance and incapable of adversely affecting pregnancy. This indicates that the cytokine and cellular environment of the uterus at the time of priming to paternal antigens in vivo provides immune-deviating signals that ensures that maternal immune tolerance is established to accommodate the conceptus.

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LEPTIN ACTION ON NEURONS IS REQUIRED FOR NORMAL PUBERTY ONSET

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The target neurons of leptin in the hypothalamus for appetite regulation have been well described. Although leptin is recognized as being permissive for normal puberty onset and fertility in mammals, whether or not this also occurs via a central action is controversial in the literature. In order to examine the hypothesis that leptin acts specifically through leptin receptors expressed on brain neurons to permit normal puberty and fertility, neuron-specific leptin receptor (*Lepr*) mutant mice were bred by crossing *Lepr-flox* mice with mice transgenic for Cre recombinase driven by a *CaMKIIα* promoter (which is expressed in forebrain neurons, but not glial cells). In this model, expression of Cre recombinase causes neuron-specific deletion of *Lepr* coding exon 17 in mice homozygous for *Lepr-flox*. Onset of puberty (date of vaginal opening and first estrous) and fertility (estrous cyclicity and number of litters born) are currently being measured in these mice. Female mutant and control (*Lepr-flox* only) mice were weaned at a similar bodyweight (9.8 ± 0.3 g). Thereafter bodyweight gain in mutant mice was approximately double that of controls so that by 5 weeks old they averaged 21.8 ± 0.6 g (vs. 16.0 ± 0.2 g in controls; $P<0.001$). Control mice exhibited vaginal opening at 32.1 ± 0.6 days old; this was followed by the first estrus (identified by presence of cornified cells in the vaginal smear) approximately 3 days later. By day 36 only a few mutant mice had undergone vaginal opening and none had displayed estrous smears. We are currently assessing estrous cyclicity in these mice, and fertility in both males and females. The phenotypes of obesity and disrupted puberty onset observed in this study are also characteristic of global leptin (*ob/ob*) and leptin receptor (*db/db*) knockout mice; therefore our data are consistent with the hypothesis that the effects leptin on both of these endpoints occurs via actions on brain neurons.

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IMPAIRED OOCYTE DEVELOPMENTAL COMPETENCE ARISES FROM DIET-INDUCED OBESITY AND CAN BE REVERSED BY PERI-OVULATORY ROSIGLITAZONE TREATMENT

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Obesity and its complications are increasingly prevalent among women of reproductive age and are associated with female infertility. Our studies in mice show diet-induced obesity (DIO), which triggers insulin resistance, dyslipidemia and symptoms of chronic inflammation, impacts ovarian function at multiple levels. Compared to female mice fed a control diet, those fed a "Western" diet (22% fat, 0.15% cholesterol) had impaired ovulation that correlated with increased bodyweight ($P=0.043$) and circulating cholesterol ($P=0.029$). Furthermore, significantly fewer embryos developed beyond the 4-8 cell stage when maintained in culture (74% vs. 53%, $P=0.040$), indicating defects in oocyte competence that correlated with elevated maternal fasting glucose ($P=0.048$). In addition, those embryos surviving to the blastocyst stage displayed abnormal allocation of cells to the inner cell mass (ICM), which was predicted by high maternal fasting insulin at the time of conception ($P=0.029$). To further identify critical cellular mediators of ovarian responses to obesity-induced insulin resistance, DIO females were treated for 4 days prior to mating with insulin-sensitizing

pharmaceuticals: 1) the PPARγ agonist rosiglitazone, 10mg/kg/day; 2) the glucose and lipid-lowering activator of AMP Kinase, AICAR, 30mg/kg/day; or 3) sodium salicylate, 50mg/kg/day. On-time development to blastocyst was significantly improved by treatment with rosiglitazone compared to vehicle-treated DIO females, effectively restoring development rates to levels comparable to females on the control diet. Treatment with AICAR or sodium salicylate did not improve blastocyst developmental timing, but all insulin-sensitizing agents improved embryonic cell allocation to the ICM. In addition, treatment with rosiglitazone, but not AICAR or sodium salicylate, modulated ovarian gene expression; in particular regulators of cellular lipid metabolism, Fabp4, Scarb1 and Cd36. These studies confirm that DIO/insulin resistance results in reduced oocyte developmental competence, and demonstrate that treatment with select insulin-sensitizing pharmaceuticals exert positive effects on oocyte quality. Improved blastocyst quality in obese females treated with rosiglitazone prior to mating indicates that PPARγ is a key target for metabolic regulation of ovarian and oocyte function.

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CENTRAL LEPTIN ALTERS POST-PRANDIAL THERMOGENESIS IN MUSCLE AND FAT OF THE SHEEP

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Brown adipose thermogenesis contributes to 30% of energy expenditure, but the extent to which muscle contributes is unknown. Our understanding of factors that control thermogenesis is limited in non-rodent species. We have studied ovariectomised ewes, since discrete tissues may be monitored with ease. We measured post-prandial heat production in fat and muscle as a determinant of thermogenesis and examined the effect of centrally administered leptin. Dataloggers were implanted into retroperitoneal (visceral) fat, subcutaneous gluteal fat (s.c.) and hind-limb muscle to measure temperature. Four ewes were fed in a window of 1100-1600 h to program post-prandial thermogenesis. After 1 week, they received an infusion of either leptin (10 μg/h) or vehicle (100 μl/h) into the lateral cerebral ventricle for 24 h (0900-0900 h); treatments were crossed over after 1 week. Food intake and core temperature was recorded. Blood samples were taken hourly (1000-1500h) to measure plasma non-esterified fatty acids (NEFA). Basal thermogenic rates (0800-1100 h) were similar in visceral fat and muscle, but lower ($P<0.05$) in s.c. fat. Leptin treatment reduced ($P<0.05$) food intake with no acute effect on basal thermogenic rate. Nor did leptin alter core body temperature in this period (0800-1100 h). Central leptin infusion markedly enhanced post-prandial thermogenesis in all sites. due to an increase in amplitude of response in s.c. fat (312.7 +/- 55.7% ;$P<0.05$), visceral fat (480 +/- 68.2 % ;$P<0.01$) and muscle (426.5 +/-72.7% ;$P<0.01$). Plasma NEFA levels were reduced ($P<0.05$) in control animals only. We report the first concurrent post-prandial excursions in thermogenesis in muscle and fat in any species and show heretofore an undescribed effect of central regulation of temperature in muscle. Leptin administration to the brain enhances the post-prandial elevation of temperature in both fat and muscle. Manipulating how the brain regulates thermogenesis in muscle is a means of controlling energy expenditure.

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LINOLEIC ACID INDUCES CA^{2+}-DEPENDENT INACTIVATION OF L-TYPE VOLTAGE-GATED CA^{2+} CHANNELS IN PRIMARY CULTURED RAT PANCREATIC BETA-CELLS

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Free fatty acids (FFAs) regulate insulin secretion in a complex pattern and may take part in the process of beta-cell dysfunction in type 2 diabetes. GPR40 was demonstrated as a specific receptor for long-chain FFAs and abundantly expressed in pancreatic beta cells (1). Voltage-gated Ca^{2+} channels in beta-cells play a major role in high glucose-induced insulin secretion. Linoleic acid (LA) was found to reduce voltage-gated K^+ currents through GPR40 receptor (2), which may lead to a membrane depolarization and opening of voltage-gated Ca^{2+} channels. The aim of present study is to test immediate effect of LA on voltage-gated Ca^{2+} currents in beta-cells and to clarify the underlying signalling system. Ca^{2+} currents in beta-cells were recorded by patch clamp and majority of Ca^{2+} currents are L-type current. LA (10 μM) significantly and reversibly inhibited the Ca^{2+} current. Methyl-linoleate, similar structure to LA but with no binding affinity to GPR40, did not alter the Ca^{2+} current. LA-induced inhibition of the Ca^{2+} current was not affected by pre-incubation of beta-cells with specific PKA inhibitor, H89 or PKC inhibitor, chelerythrine. The inhibitory effect of LA on the Ca^{2+} current was, however, completely abolished by pre-incubation with thapsigargin, a reagent depleting $InsP_3$-sensitive Ca^{2+} stores. LA also increased level of $[Ca^{2+}]i$ in beta cells and such increase in $[Ca^{2+}]i$ was blocked by thapsigargin pre-treatment of beta-cells but not by blockade of Ca^{2+} channels. Methyl-linoleate did not induced the increase in $[Ca^{2+}]i$. It is concluded that LA stimulates $InsP_3$-sensitive Ca^{2+} release from intracellular Ca^{2+} stores through GPR40 receptor leading to an inhibition of L-type Ca^{2+} current through Ca^{2+}-dependent inactivation of Ca^{2+} channels. Rapid increase in $[Ca^{2+}]i$ by FFAs leads to an increase in insulin secretion.

Supported by NHMRC and Eli Lilly Australia.

(1) Itoh et al, Nature 422:173-176; 2003
(2) Feng et al, Endocrinology 147:674-682; 2006

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PROGRAMMING OF ADULT HYPOTHALAMIC LEPTIN RESISTANCE BY FETAL GLUCOCORTICOID EXCESS IN THE RAT

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Fetal glucocorticoid excess programs hyperleptinemia in adult rat offspring without any apparent change in adiposity, suggestive of relative leptin resistance. In this study we tested the hypothesis that hypothalamic expression of leptin-regulated signalling molecules is unaffected by programmed hyperleptinemia. Support for this hypothesis would be consistent with programmed leptin resistance. We also determined whether programmed hyperleptinemia is associated with increased plasma leptin binding activity, since this can restrict leptin transfer to the extravascular space. Offspring of control (Con) and dexamethasone (Dex)-treated mothers (0.75 μg/ml drinking water, from day 13 to term) were cross-fostered within 24 h of birth to mothers on a standard or high-omega-3 (Hn3) diet. Previous studies show that this dietary manipulation prevents development of adult hyperleptinemia. Plasma and brains were obtained from male offspring at 12 months of age (n=6-8), and the hypothalamus isolated by dissection. Hypothalamic neuropeptide Y (NPY), agouti-related peptide (AgRP), proopiomelanocortin (POMC), cocaine-amphetamine-regulated transcript (CART), suppressor of cytokine signalling-3 (SOCS-3) and the leptin receptor (Ob-Rb) were measured by quantitative RT-PCR. Peripheral plasma leptin binding was assayed by incubation of plasma with radiolabelled leptin followed by non-denaturing gel electrophoresis. Despite marked hyperleptinemia, prenatal Dex increased hypothalamic AgRP expression, an effect opposite to that expected in response to elevated leptin, and had no effect on hypothalamic POMC, CART, NPY or SOCS-3 mRNA expression. Prenatal Dex also had no effect on hypothalamic Ob-Rb expression and peripheral plasma leptin-binding activity, although the latter was reduced by the Hn3 diet in offspring exposed to prenatal Dex. In conclusion, these data suggest that hypothalamic expression on leptin-regulated neuropeptides is unaffected by sustained hyperleptinemia programmed by prenatal Dex. This apparent leptin resistance may reflect reduced leptin transport across the blood-brain barrier and/or disturbances in signalling downstream of Ob-Rb. Thus, the programmed phenotype is likely to be more susceptible to diet-induced obesity.

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MATERNAL DEXAMETHASONE TREATMENT PROGRAMS THE ADIPOCYTE PHENOTYPE IN ADULT OFFSPRING

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Adverse fetal environments can lead to a predisposition for the metabolic syndrome, including diet-induced obesity and related disorders. Previously we demonstrated that adult offspring of mothers treated with dexamethasone (Dex) develop hyperleptinemia without increased adiposity, and that this effect is prevented if offspring are raised on a high omega-3 fatty acid (Hn3) diet. In the present study we characterised the adipocyte phenotype of these animals. Offspring of control (Con) and Dex-treated mothers (0.75 μg/ml drinking water, from day 13 to term) were cross-fostered to mothers on a standard (Std) or Hn3 diet within 24 h of birth. Offspring remained on these diets post-weaning, and plasma and retroperitoneal fat were obtained from 6-month old males (n=5-8 per group). Adipose mRNA expression for the glucocorticoid receptor (GR), 11β-HSD1, leptin, adiponectin, Glut-4, the peroxisome proliferators-activated receptors (PPARs), uncoupling protein-2 (UCP2) and TNFα were determined by quantitative RT-PCR. Plasma levels of TNFα, interleukin (IL)-1β, and IL-6 were measured by EIA and adipose morphology (adipocyte size and % multilocular cells) was assessed by stereology. Offspring of Dex-treated mothers had elevated adipose expression of Glut-4 (↑13-fold), GR (↑3-fold), TNFα (↑3-fold), PPARα (↑36%) and leptin (↑16%), reduced UCP2 (↓88%), but increased (↑3.5-fold) % multilocular cells. Only elevation of leptin was prevented by the Hn3 diet. PPARδ expression was increased (↑65%) by Hn3 which also reduced adipocyte size (↓24%) regardless of prenatal treatment. Dex offspring raised on the Std (but not the Hn3) diet had higher plasma IL-1β (↑66%) and IL-6 (↑24%), whereas plasma TNFα was elevated (↑77%) in all Dex offspring. In conclusion, this study shows that fetal glucocorticoid excess has marked programming effects on the adipocyte phenotype, with likely consequences for substrate metabolism and cytokine production. Partial prevention of this phenotype by Hn3 suggests that dietary manipulations could limit adverse effects of the programmed phenotype.

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CXCL12 (STROMAL CELL-DERIVED FACTOR-1) SECRETION BY PREADIPOCYTES IS ENHANCED BY SHORT-CHAIN FATTY ACIDS (SCFAS), ACTING THROUGH A G PROTEIN-COUPLED RECEPTOR (GPR41)

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CXCL12 is involved in diabetic retinopathy, and decreased secretion in diabetes contributes to defective vascular progenitor cell mobilisation, leading to macrovascular complications. Adipocytes could be an important source of CXCL12 and other chemokines. The SCFA butyrate stimulates, while other histone deacetylase (HDAC) inhibitors (valproate and trichostatin) inhibit, adipogenesis.

3T3-L1 cells were grown to confluence and studied as preadipocytes or differentiated adipocytes. Butyrate increased CXCL12 mRNA 5-fold ($p < 0.001$) and protein secretion 2-fold ($p < 0.001$) in preadipocytes. CXCL12 was expressed in adipocytes, but butyrate had modest effects on gene expression and did not affect protein secretion.

CXCL12 mRNA and protein were not increased in preadipocytes by octanoate or by valproate or trichostatin. SCFAs increased CXCL12 secretion in preadipocytes, with potency propionate > butyrate > acetate consistent with action through the GPR41.

Pertussis toxin (5 ng/ml), which inhibits receptor-G protein interaction, was without effect alone but abolished the stimulatory effect of SCFA on CXCL12 expression.

SCFA did not affect VEGF mRNA, but decreased VEGF secretion in preadipocytes. In adipocytes, butyrate increased VEGF mRNA and protein (41% and 22%, $p < 0.01$ and $P < 0.05$, respectively) whereas HDAC inhibitors decreased VEGF secretion. Hypoxia increased VEGF mRNA ($p < 0.001$), but decreased CXCL12 ($p < 0.001$). MCP-1 was predominantly expressed in preadipocytes, and up-regulated by SCFA ($p < 0.001$). mRNA for CXCR4, the receptor for CXCL12 was not detected in preadipocytes or adipocytes, but was present in monocytic cells. GPR41 mRNA was detected in preadipocytes and adipocytes. GPR43, the other known SCFA receptor, was only present in adipocytes.

CXCL12 may mediate interaction of adipocytes with immune and vascular cells, Regulation by SCFA and hypoxia differs to that of VEGF. Increased preadipocyte differentiation in obesity may contribute to decreased CXCL12 and this may be partly reversed by functional foods which increase SCFA.

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THE ESTROGENIC COMPONENT OF TIBOLONE REDUCES ADIPOSITY IN FEMALE AROMATASE KNOCKOUT (ARKO) MICE - A MODEL OF MENOPAUSE

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Tibolone (ORG OD14), a hormone replacement therapy (HRT), has estrogenic, progestogenic &/or androgenic activity depending on the tissue. The Aromatase knockout (ArKO) mouse, with its inability to synthesize endogenous estrogens, provides a valuable model of menopause to study the multiple properties of tibolone. The aim of this study was to determine the consequences to the adipose and lipid profiles of separate and combined treatments with each of the ovarian hormones mimicked by tibolone.

Ovariectomised ArKO mice were orally administered tibolone (2µg/g), ethinyl estradiol (EE) (0.1µg/g), a pure progestogen (ORG2058) (1µg/g), EE + ORG2058, or vehicle once daily for 6 weeks. Dihydrotestosterone (DHT) was administered via slow release subcutaneous pellet (0.5mg/pellet; Innovative Research of America).

Tibolone treatment reduced gonadal fat (GF), infra-renal (IF), and brown adipose tissue (BAT) masses, and GF adipocyte numbers (AN) compared to vehicle-treated controls (86% $p<0.01$, 83% $p<0.01$, 60% n.s., 69% n.s. reduction respectively). Similar reductions in adiposity were observed for EE-alone treatment compared to controls (GF 83% $p<0.01$, IF 82% $p<0.05$, BAT 47% n.s., AN 81% $p=0.059$.). In contrast, adipose tissue mass tended to increase following ORG2058-alone treatment compared to vehicle-treated controls (GF 29% n.s., IF 44% $p<0.05$, BAT 32% n.s., AN 54% $p=0.053$). However this effect was counteracted by the restoration of EE treatment (EE + ORG2058) (GF 77% $p<0.05$, IF 80% $p<0.01$, AN 46% $p<0.05$ reduction compared to their corresponding ORG2058-alone treatment groups). DHT treatment did not impact adipose tissue mass when compared to controls. Lipid metabolism gene expression, circulating lipids and adipokine profiles are currently being assessed.

These results demonstrate that it is the estrogenic and not the androgenic or progestogenic components in tibolone that are the primary effectors in reducing adiposity within this model. These data further confirm the efficacy of tibolone as an HRT to reduce adipose tissue accumulation following menopause and also show that aromatisation of tibolone is not required to elicit these estrogenic effects.

n.s. non-significant; statistics: ANOVA; post-hoc Tukey test.

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EFFECT OF PLACENTAL RESTRICTION ON CIRCULATING LEPTIN AND ITS RELATIONSHIP TO FEEDING ACTIVITY AND ADIPOSITY IN THE YOUNG LAMB.

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Low birth weight and infant catch-up growth predict increased adiposity in children and adults. Why is unclear, but in individuals who are of small at birth and intrauterine growth restricted, plasma leptin is low at birth, then increased, including relative to adiposity, in adulthood. As leptin is secreted from adipocytes and regulates body energy intake and expenditure, early life programming of leptin resistance may partly mediate perinatally induced alterations in adiposity. Placental restriction (PR) is a major cause of intrauterine growth restriction and we have shown that in sheep, PR reduces size at birth, causes catch-up growth, and increases feeding activity and adiposity by 6 weeks of age[1]. We therefore hypothesised that PR would increase plasma leptin and alter its relationship with feeding activity and adiposity in the young lamb. Placental growth was restricted by removal of endometrial caruncles prior to mating[1]. Body size, plasma leptin (day 5 to 40), feeding activity in terms of numbers of suckling events and duration for 2.5 hours following one hour fasting (day 15)[1], and visceral fat (day 45), were measured in 15 control and 12 PR sheep. PR reduced size at birth, and increased postnatal growth rate up to and adiposity at day 45. Plasma leptin decreased with age and was increased overall and at day 40 by PR. Suckling events, in terms of total number and relative to body weight, correlated negatively with plasma leptin in control, but not in PR lambs. Plasma leptin correlated positively with visceral adiposity in absolute and relative to body weight terms and similarly in control and PR lambs. These findings show that high circulating leptin predicts reduced suckling attempts in the normal lamb and that placental restriction abolishes this, and increases plasma leptin and adiposity. This suggests that PR programs resistance to appetite regulation by leptin postnatally, leading to early onset obesity.

(1) De Blasio, MJ et al (2007) Am J Physiol. 292: R875.

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INCREASED ADIPOSITY IN OFFSPRING OF MURINE DIABETIC PREGNANCY IS DUE TO ALTERATIONS IN FUEL METABOLISM.

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Background: The origins of obesity lie in the complex interaction between genes, diet/lifestyle and the early developmental-environment. Offspring of diabetic-pregnancy are at higher risk of obesity than non-diabetic-pregnancy offspring. We use a novel-animal-model of diabetic-pregnancy, the ß-cell-specific-ARNT (Aryl-hydrocarbon-Receptor Nuclear-Translocator) knockout (ß-ARNT) mouse to examine the effects on offspring with and without a genetic-predisposition to diabetes.

Methods: ß-ARNT and floxed-control females were mated with floxed-control and ß-ARNT males respectively, giving mixed litters of ~50% ß-ARNT and ~50% floxed-control mice. Male offspring were studied. Food-records, DEXA and indirect-calorimetry were performed.

Results: Non-pregnant ß-ARNT females are mildly glucose-intolerant owing to defective insulin secretion. In late-pregnancy, ß-ARNT but not floxed-control dams show predictable worsening of glucose-tolerance with peak glucose of 21.8 versus 14.4mmol/L ($p<0.01$), confirming them as a diabetic-pregnancy-model. From 7-weeks, ?-ARNT offspring from diabetic-pregnancy were significantly heavier than those from non-diabetic-pregnancy, despite eating less ($p<0.05$). As expected, ?-ARNT diabetic-pregnancy-offspring had significantly higher body-fat-percentage. By contrast, floxed-control offspring from diabetic-pregnancy showed only a trend to be heavier than non-diabetic-pregnancy offspring. This may be secondary to increased energy-expenditure, which was associated with higher activity-levels. Despite the increased energy-expenditure and activity, these mice had still had significantly increased body-fat-percentage. Diabetic-pregnancy offspring of both genotypes had an increased postabsorptive respiratory exchange ratio (RER), indicative of propensity to store rather than burn fat. Area-under-curve for maternal-GTT during pregnancy was highly significantly correlated with offspring weight ($r=0.53$, $p=0.001$) and offspring RER ($r=0.46$ $p=0.01$).

Conclusion: Diabetic-pregnancy leads to increased offspring adiposity, and the effects are modified by offspring genotype. This study demonstrates the interaction between the early developmental environment and genotype in producing the obese phenotype. These data may help us to understand why some humans are at greater risk of obesity than others, despite reducing their energy consumption, a phenomenon previously attributed to genetic risk alone.

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OESTROGEN EFFECTS ON PROLACTIN INDUCED STAT SIGNALLING

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Oestrogen and prolactin (PRL) interact to regulate breast development in puberty and pregnancy, and following parturition oestrogen can be used to suppress PRL-induced lactation. However, little is known about the molecular mechanisms by which these two hormones interact. The aim of this study was to investigate the effects of oestrogen on PRL-induced signalling.

Human embryonic kidney (HEK) 293, or HC11 mouse mammary epithelial cells were transfected with a luciferase reporter construct containing either 5 repeats of the LHRE STAT5 binding element, or the native β-casein promoter. The cells were also transfected with PRL receptor (PRLR), oestrogen receptor (ER) α, and STAT5a as required. Cells were treated overnight with PRL (0-1000ng/ml) and 17β-oestradiol (E2; 0-100nM) before lysates were collected and luminescence measured.

E2 (100nM) had no independent effect on LHRE reporter activity, but reduced the PRL-induced response from 5.5±0.2- to 2.8±0.3-fold in HEK293 cells ($p<0.05$). The inhibitory effect of E2 was dose-dependent, present at 1pM ($p<0.05$), and maximal at 1nM. The effect of E2 required the presence of ERα, and was abrogated by pre-treatment with anti-oestrogen ICI182780 (1mM). Similar results were found in HC11 cells, and using the native β-casein promoter reporter.

In summary, oestrogen inhibited PRL-induced STAT signalling in two cell lines via an ER- dependent mechanism. This effect may occur via induction of suppressors of cytokine signalling (SOCS), as previously described for oestrogen inhibition of GH action (1). Further studies will also determine the effects of oestrogen on expression of the milk protein, β-casein, in HC11 mammary cells.

(1) Leung et al. (2003) Estrogen inhibits GH signaling by suppressing GH-induced JAK2 phosphorylation, an effect mediated by SOCS-2. PNAS 100(3):1016-21.

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REGULATION OF GROWTH FACTOR RECEPTOR GENE EXPRESSION IN HUMAN CANCER BY MIRNAS

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MicroRNAs (miRNAs) are endogenous, non-protein coding small RNAs that repress gene expression at the posttranscriptional level. Aberrant expression of miRNAs has been described in a range of human cancers and modulation of miRNA expression can regulate growth or survival of some tumor cells. Thus a major challenge is to understand the processes by which miRNAs might participate in tumorigenesis. To date, few direct targets of miRNAs have been identified in cancer cells. The epidermal growth factor receptor (EGFR) and erbB-2/HER-2 growth factor receptors (GFRs), which are overexpressed in many human cancers and involved in tumor

development and progression, are major therapeutic targets. We have focussed on interactions between human miRNAs and these two GFRs. Each of their 3'-UTRs contain putative targets for specific miRNAs, and in in vitro assays we found that treatment of cells with specific miRNA precursors significantly reduces expression of the corresponding receptor in a range of cancer cells, including lung, breast, prostate and glioblastomas. Using reporter assays, we validated the interaction in a range of human cancer cells. Furthermore, expression of specific miRNAs in cancer cell lines induced a reduction in cell proliferation and a cell cycle block. Using gene arrays we have identified additional direct targets that play a role in GFR signaling, suggesting that some miRNAs may coordinately directly regulate expression of several components of the GFR signaling pathway. Taken together, these data indicate that miRNAs can be potent regulators of GFR expression in human cancer, and suggest a possible therapeutic role for these miRNAs to modulate GFR signaling and tumor growth.

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RAPAMYCIN TREATMENT OF A BOY WITH PROTEUS SYNDROME AND A GERMLINE *PTEN* MUTATION

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Germline *PTEN* mutations are associated with hamartomatous disorders including Cowden, Bannayan-Riley-Ruvalcaba and Proteus syndromes, collectively described as the *PTEN* Hamartoma Tumour syndrome. Proteus syndrome is a rare sporadic disorder manifesting as overgrowth of multiple tissues leading to progressive skeletal deformities, invasive lipomas, vascular malformations, and benign and less commonly malignant tumours. *PTEN* mutant cells exhibit constitutive activation of the phosphatidylinositol 3-kinase (PI3-K)/mammalian target of rapamycin (mTOR) signalling pathway. The molecular target drug rapamycin, also known as sirolimus, inhibits mTOR signalling by binding simultaneously to FKBP12 (FK506-and rapamycin-binding protein) and the FKBP12 binding domain of mTOR kinase. We describe a 6 year old boy diagnosed with Proteus syndrome and the germline *PTEN* mutation c.507delC who has undergone rapamycin therapy. Computed tomography (CT) scans prior to commencement of rapamycin treatment at 2 years and 2 months and again at 14 months after the initiation of treatment showed a marked reduction in soft tissue masses in the anterior mediastinum and the right side of the pelvis, as well as a reduction in size of the mesenteric lymph nodes. After a 3 month period of cessation of the drug from 17 months into treatment, CT sc ans showed an increase in the size of soft tissue masses and mesenteric lymphadenopathy. Elevated serum insulin-like growth factor binding protein-2 (IGFBP-2) levels declined during rapamycin treatment and peaked in periods off rapamycin, suggesting this biomarker may be used to monitor treatment. In PC3 *PTEN*-null cells transiently transfected with wild-type or mutant *PTEN*, rapamycin inhibited basal and epidermal growth factor stimulated phosphorylation of S6 kinase, an mTOR target. This case study provides a rationale for rapamycin therapy in patients with disorders in the *PTEN* Hamartoma Tumour syndrome spectrum, and other disorders with elevated PI3-K/mTOR signalling, and for further investigation of IGFBP-2 serum levels in the management of these patients.

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ANTI-PROLIFERATIVE AND PRO-DIFFERENTIATION EFFECTS OF FGF-2 ON SK-N-MC CELLS INVOLVES REGULATION OF ID GENES AND INHIBITION OF EMT-LIKE MECHANISMS

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An early event in neuroblastoma (NB) pathogenesis is the arrested differentiation of neuroblasts. These neural crest (NC) derived embryonic tumor cells have switched from an epithelial phenotype to a highly mobile mesenchymal phenotype, a process known as epithelial–mesenchymal transition (EMT). We aimed to investigate the cellular and molecular mechanisms involved in the inhibition of neuroblast differentiation and enhancement of motility.

We recently demonstrated that FGF-2 promotes NB cell differentiation and over-rides their mitogenic response to growth factor IGF-I. Certain genes have been implicated in the regulation of neuronal cell differentiation, including the transcriptional regulator ID genes and genes involved in EMT-like events. Whether these genes mediate FGF-2 differentiation and/or growth arrest of NB cells is unknown.

SK-N-MC cells were cultured in the presence or absence of FGF-2 (50ng/ml) and/or IGF-I (100ng/ml) for up to 48h. Neuronal-GAP43 stain confirmed NB cell differentiation. FGF-2 inhibition of IGF-I mediated SK-N-MC cell proliferation was also confirmed. Analysis by FACS of SK-N-MC cell cycle, under the above culture conditions, is in progress.

Extracted total RNA was analysed for ID1-3 gene expression by real time (Q) PCR. ID2 was strongly induced by FGF-2, while ID1and ID3 where slightly down-regulated. Concurrently, FGF-2 induced nucleic accumulation of ID2 protein, while ID1 and ID3 remained cytoplasmic. The known ID2-regulated gene Neuro-D6, a neuronal differentiation marker, was dramatically up-regulated, and this effect was blunted by ID2 siRNA.

We then investigated whether FGF-2-induced differentiation of SK-N-MC cells involves their transition/conversion from mesenchymal to epithelial cell phenotype (MET). Using Q-PCR, a range of genes involved in cell-cell adhesion and maintenance of

the epithelial phenotype (E-cadherin, Cadherin-6), cell-matrix interactions (Col 4-A2; FN; MMPs; TIMPs), tumor suppression (MTSS1; KiSS1) and Snail-1 were found upregulated.

In conclusion, we have shown that FGF-2 activates a complex gene expression program in NB cells consistent with the progression of neuroblasts toward differentiation and suppression of their metastatic phenotype. Our findings thus point to novel therapeutic targets for this devastating childhood cancer.

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THE INFLUENCE OF GENDER AND TESTOSTERONE ON THE RESPONSE TO GH OF IGF AXIS AND COLLAGEN MARKERS IN YOUNG RECREATIONAL ATHLETES: A DOUBLE-BLIND PLACEBO-CONTROLLED STUDY.

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Serum IGF axis proteins and collagen peptides increase in response to GH, therefore are useful markers of GH abuse; however little is known in young healthy adults about the influence of gender, and of combined treatment with testosterone (T). The aim was to investigate in young recreational athletes whether the response of IGF axis and collagen markers to GH differed between men and women, and with combined administration of T.

Recreational athletes were administered GH (2 mg sc/day) and/or T (250 mg Sustanon IM/week) for 8 weeks followed by 6 weeks washout in a double-blinded placebo-controlled study. Women (28.7 ± 5.8 yrs) were randomized to GH (n=17) or placebo (n=16). Men (27.6 ± 5.6 yrs) were randomized to GH, T, GH+T or placebo (n=16 in each group). Serum IGF-I, IGFBP-3, ALS, PINP, ICTP and PIIINP were measured by immunoassay in samples collected at baseline and during treatment and washout. Statistical analysis of treatment effects was performed both at a single time point (week 8) and at all time points using a linear mixed effects model on log transformed data.

The markers increased significantly in response to GH compared to placebo (Table). When considered across all time points, the GH response was significantly greater ($P<0.001$) in men than in women for all the markers. In men, treatment with T alone did not show a significant effect on IGF markers and combined treatment (GH+T) did not show a significant change compared to the response to GH alone. However in response to T alone, PIIINP increased significantly in men, and combined treatment (GH+T) significantly increased the response to GH alone. All collagen markers remained elevated longer than the IGF axis markers following withdrawal of GH.

Percentage change at week 8 compared to baseline, means (SE)

	Men				Women	
	Placebo	GH	T	GH + T	Placebo	GH
% Δ IGF-I	3 (7)	137 (23)	9 (4)	160 (24)	3 (8)	86 (12)
% Δ IGFBP-3	4 (3)	29 (5)	-1 (4)	24 (3)	-2 (3)	33 (8)
% Δ ALS	5 (3)	39 (9)	2 (6)	40 (6)	-3 (3)	24 (7)
% Δ PINP	-9 (4)	127 (25)	28 (11)	185 (27)	-2 (4)	85 (23)
% Δ ICTP	4 (4)	159 (24)	22 (4)	196 (19)	-2 (4)	87 (16)
% Δ PIIINP	5 (4)	239 (39)	70 (17)	419 (56)	-1 (4)	155 (26)

In summary, the response of all markers differed between men and women, and testosterone enhanced the GH-induced increase in PIIINP. We conclude that these markers of GH abuse are less sensitive in women and that the use of serum collagen markers extends the window of detection of GH doping. The sensitivity of the collagen marker PIIINP is increased by co-administration of testosterone.

Funding support from the World Anti-Doping Agency and Australian Government Anti-Doping Research Program. GH was provided by Novo Nordisk and testosterone by Organon.

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EFFECTS OF BOVINE SOMATOTROPHIN ON CIRCULATING IGF-1 CONCENTRATION AND MILK PRODUCTION IN RED DEER

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To determine the role of milk production on growth performance of their calves, lactating red deer hinds (n = 10) were treated in summer with bovine somatotrophin (bST, 54 mg s.c. as a single dose fortnightly for 8 weeks, then 108 mg fortnightly for 8 weeks) or saline solution. Daily milk production, based on the 6 hour production of 4 hinds/group, was assessed on 5 occasions during the 4 months treatment period. Plasma IGF-1 concentration (mean ± S.E.M.) was elevated ($P < 0.001$) in a positive dose-related manner by bST treatment (from 73.4 ± 9.47 to 101.3 ± 11.21 and 165.4 ± 11.70 ng/ml at 24 h after 54 mg and 108 mg bST, respectively) but there was no effect on milk production or calf growth. In another study, after removal of calves and treatment with bST (54 mg s.c. as a single dose, then 108 mg likewise 2 weeks later, n = 10) or saline solution (n = 9), deer hinds were machine milked twice daily for 3 weeks during January to provide a direct measure of milk production. Daily milk yield (mean ± S.E.M.) increased from 695 ± 56.6 and 584 ± 54.3 (treated and control) to 927 ± 104.9 and 721 ± 57.6 ml, respectively, at 7 days after 54 mg and to 1202 ± 93.1 and 877 ± 59.3 ml, respectively, at 7 days after 108 mg bST; the latter increase in yield being significantly ($P = 0.013$) greater in treated hinds. Plasma IGF-1 concentration (mean ± S.E.M.) increased ($P < 0.001$) from 75.6 ± 8.48 to 118.8 ± 13.90 and 295.8 ± 25.48 ng/ml at 24 h after 54 mg and 108 mg bST, respectively. These results support the concept that milk production of deer hinds is dependent on the level of demand from their calves; thus red deer calves do not avail themselves of potential milk production from their dams. The results also show a dose-dependent effect of bST on circulating IGF-1 concentration, indicating that IGF-1 may mediate the potential for increases in milk production in red deer hinds.

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DEXAMETHASONE ADMINISTRATION INHIBITS SKELETAL MUSCLE EXPRESSION OF THE ANDROGEN RECEPTOR AND IGF-1 – IMPLICATIONS FOR STEROID-INDUCED MYOPATHY.

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Glucocorticoids are a well recognised cause of muscle weakness. Myopathy is a specific finding in patients with Cushing's syndrome, while patients treated with steroids for inflammatory and other medical conditions also frequently develop myopathy. We hypothesised that acute administration of the potent glucocorticoid dexamethasone would result in down-regulation of the androgen receptor and also of the IGF-1 signalling pathway. 24 subjects (12 men and 12 women) underwent venous blood sampling and biopsy of vastus lateralis before and after administration of dexamethasone 4mg/day for 4 days. Serum testosterone and IGF-1 were measured in both sexes. Quantitative RT-PCR was undertaken on the muscle specimens to measure mRNA levels of the androgen receptor, IGF-1, IGF-1 receptor and myostatin, with levels calibrated against a commercially obtained skeletal muscle cDNA library. At baseline, testosterone was higher in males as expected (15.0±1.3 vs 1.8±0.5 nmol/L, $P<0.001$), but relative expression of the androgen receptor was similar (male 1.63±0.37 vs female 1.57±0.30). Serum IGF-1, and skeletal muscle expression of IGF-1 and IGF-1 receptor were also similar between male and female subjects. Following dexamethasone, there was a significant down-regulation of skeletal muscle androgen receptor (1.60±0.23 vs 1.11±0.16, $P<0.05$) and IGF-1 (1.72±0.29 vs 1.06±0.14, $P<0.05$) mRNA levels, but no change in mRNA expression of the IGF-1 receptor or myostatin. Serum testosterone fell significantly in both sexes (male: 15.0±1.3 vs 11.3±1.2 nmol/L, $P<0.01$, female: 1.8±0.5 vs 0.5±0.1 nmol/L, $P<0.05$). In contrast, serum IGF-1 rose significantly after dexamethasone (18.5±0.9 vs 23.6±1.5 nmol/L, $P<0.001$), an equal effect observed in both sexes. Steroid myopathy may result at least in part from relative androgen deficiency at two levels, reduced circulating testosterone and local downregulation of the androgen receptor in skeletal muscle. Further research is indicated to investigate the effect of supplemental androgen therapy on muscle strength in patients on long term glucocorticoids.

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DOES GROWTH HORMONE AND TESTOSTERONE SUPPLEMENTATION IMPROVE PHYSICAL PERFORMANCE? A DOUBLE-BLIND PLACEBO-CONTROLLED STUDY IN RECREATIONAL ATHLETES.

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GH and testosterone (T) are widely abused performance-enhancing substances in sport. However whether GH alone or in combination with T enhances performance in young healthy adults is unclear. The aim was to study the effects of GH and testosterone supplementation on physical performance.

In a double-blind study of recreational athletes (exercising >2 h/wk for > one year, aged 27.9 ± 5.7 y, mean ±SD), 64 men were randomised to 8 weeks' treatment with placebo, GH (2 mg/d), T (250 mg IM Sustanon/wk, 5 wks) or combined treatments, and 33 women to placebo or GH (2 mg/d). Physical performance was measured before treatment and at 8 wks by a) a sub-maximal predictive cycle test for VO_2max, b) dead lift dynamometry for strength, c) vertical jump height for power, and d) sprint cycle

ergometry (Wingate test) for anaerobic work capacity. Statistical analysis was performed using repeated measure ANOVA and significance determined after Bonferroni's correction.

Results are expressed as % change from baseline (mean±SEM).

	Men				Women	
	Placebo	GH	T	GH+T	Placebo	GH
VO_2 max	0.9±2.5	0.4±3.5	4.4±3.1	3.6±3.5	0.0±2.8	3.9±3.3
Dead lift	6.9±2.9	7.8±3.2	6.5±4.7	1.6±4.4	4.9±3.8	1.2±2.5
Jump height	2.7±1.5	2.9±1.5	1.8±1.3	3.0±2.4	1.3±2.7	4.6±2.1
Wingate	0.8±1.7	5.8±1.7	4.9±1.6	9.6±2.2*	3.0±2.2	5.5±2.1

*: $p<0.05$ compared to placebo.

In men, when compared to placebo, GH alone did not significantly change VO_2 max, dead lift, jump height or an aerobic work capacity. T alone also did not significantly change any of the four measures of performance compared to placebo. Combined treatment significantly increased anaerobic work capacity. In women, GH did not significantly change any of the measures of physical performance compared to placebo.

We conclude that in young healthy adults, short term GH or T supplementation in the doses used does not significantly improve physical performance, while combined treatment enhances anaerobic work capacity in men. The effects of higher doses for longer duration on performance merit investigation.

Funding support from the World Anti-Doping Agency and the Australian Government Anti-Doping Research Program. GH was provided by Novo Nordisk and testosterone by Organon.

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ENDOCRINE REGULATION OF ENERGY METABOLISM BY THE SKELETON

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The regulation of bone remodeling by an adipocyte-derived hormone implies that bone may exert a feedback control of energy homeostasis. To test this hypothesis we looked for genes expressed in osteoblasts, encoding signaling molecules and affecting energy metabolism. We show here that mice lacking the protein tyrosine phosphatase OST-PTP are hypoglycemic and are protected from obesity and glucose intolerance because of an increase in □-cell proliferation, insulin secretion, and insulin sensitivity. In contrast, mice lacking the osteoblast-secreted molecule osteocalcin display decreased □-cell proliferation, glucose intolerance, and insulin resistance. Removing one Osteocalcin allele from OST-PTP-deficient mice corrects their metabolic phenotype. Ex vivo, osteocalcin can stimulate CyclinD1 and Insulin expression in b-cells and Adiponectin, an insulin-sensitizing adipokine, in adipocytes; in vivo osteocalcin can improve glucose tolerance. By revealing that the skeleton exerts an endocrine regulation of sugar homeostasis this study expands the biological importance of this organ and our understanding of energy metabolism.

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HORMONAL CONTROL OF GESTATION AND PARTURITION IN VIVIPAROUS LIZARDS

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The evolution of viviparity requires loss of the egg shell, loss of nesting behaviours, elaboration of placentae and, importantly, evolution of the endocrine system so as to support gestation and parturition. Among squamate reptiles, in which the ancestral condition is oviparity, viviparity is thought to have evolved 105 times in 34 different lineages. Viviparous squamates therefore represent diverse, alternative evolutionary pathways to viviparity, and provide opportunities for examining whether fundamental endocrine control pathways are conserved amongst vertebrates. Unlike mammals, they exhibit a wide spectrum of placental complexity and marked variation in the proportion of embryonic nutrition supplied by yolk versus placenta. While gestation is generally assumed to be supported by the corpus luteum, patterns of plasma progesterone concentrations during gestation vary markedly between species, and the possible role of progesterone in maintaining the reptilian placenta remains elusive. As ectotherms, reptiles are particularly constrained by environmental temperatures: if the active season is short, vitellogenesis, gestation and parturition may not be completed in a single season. At the proximate level, temperature may modulate the endocrine cascade that initiaties parturition, allowing maternal control of the timing of parturition. Viviparous squamates, with their diverse range of reproductive strategies, provide us with an array of model species with which we can ask fundamental questions about the evolution of viviparity. As one example, Niveoscincus microlepidotus is an alpine lizard with an unusual biennial reproductive cycle: there is a protracted gestation period of 14 months, and embryonic development is completed several months before parturition. This species provides a particularly useful model for examining the endocrine control of parturition in squamates, and the possibility of embryo-maternal signaling in viviparous reptiles.

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GENETICS OF STEM CELLS – HOW FRUITFLIES CAN ASSIST REPRODUCTIVE TECHNOLOGY

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Stem cell populations respond to a variety of regulatory cues that control rates of cell survival, regeneration and differentiation in order to balance the renewal of the stem cell pool with a requirement for tissue replacement. We have been screening for genes that affect stem cell proliferation / differentiation and identified a requirement for the translational repressor, Musashi (Msi) for maintaining germline stem cells in the testis.

We are using the *Drosophila* testis to model stem cell behaviour within an *in vivo* niche. The *Drosophila* male germ line is established by a small number of stem cells that produce differentiating progeny throughout adult life. In contrast to many renewing tissues, the identity of the germline stem cells in *Drosophila* and their position in the gonad, and physical relationship to surrounding somatic cells are known.

Loss of *msi* causes premature differentiation of germline and somatic stem cells as well as causing a breakdown of the structure of a component of the stem cell niche, the hub cells. Loss of Msi function causes germline cells in the stem cell niche to prematurely express the differentiation marker, Bam. Murine Msi homologues are expressed in germline cells suggesting evolutionary conservation of Msi function. We have also identified that different splice forms of the RNA export regulator, Held-out-wings (How), have opposing effects on stem cell maintenance.

Identification of evolutionarily conserved molecules and mechanisms that regulate stem cell behaviour will allow manipulation of these cells in germ cell transplantation and fertility control.

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SEX IN DRAGONS: A THEORETICAL FRAMEWORK FOR THE EVOLUTION OF SEX DETERMINATION IN REPTILES.

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In contrast to endotherms (mammals and birds), ectothermic vertebrates (reptiles, amphibians, and fish) display remarkable diversity in sex determining mechanisms. For instance, reptile species exhibit genotypic sex determination (GSD) with either male (XX:XY) or female (ZZ:ZW) heterogamety, or alternatively, one of three different patterns of temperature-dependent sex determination (TSD), where the environmental temperature experienced by eggs in the nest determines their sex. Recently, the traditional view that GSD and TSD are distinct and mutually-exclusive modes of sex determination in reptiles has been challenged. I expand upon recent work in Australian lizards to explore the interaction of the two mechanisms within a single population. I present a mechanistic model for the interaction of temperature and genes in sex determination, and build this into a conceptual framework to explain the molecular and chromosomal changes occurring during evolutionary transitions between TSD and GSD. This theory can account for much of the variation in sex determining mechanisms exhibited by reptiles (and potentially other non-mammalian vertebrates), and also reveals a simple and novel route for evolutionary transitions between male heterogamety, female heterogamety, and TSD.

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ESTROGEN IS NOT ESSENTIAL FOR FULL ENDOMETRIAL RESTORATION IN A MOUSE MODEL.

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Dogma regarding endometrial regeneration following menses is that estrogen (E)-primed proliferation is required for re-establishment of full thickness endometrium and that while it may not be required for initial re-epithelialization, it is essential for stromal renewal. Lack of suitable animal models has limited functional studies on menstruation and endometrial repair. This study aimed to determine if E is essential for endometrial restoration in a mouse model.

In the model[1,2] one uterine horn of each ovariectomised (ovx) mouse is decidualized and progesterone (P) support withdrawn. Endometrial breakdown is complete by 24h and repair by 48h, closely resembling the human endometrium at menses. In this model, which lacks ovarian E, the endometrium is rapidly and fully restored. However, estrogenic influences from extra-ovarian sources (diet and fat) remain: dietary E has significant effects on uterine weight[3].

Two groups of mice were used: control (n=13); ovx + normal diet + vehicle, and E-free (n= 16); ovx + soy-free diet + letrozole (20mg/day after P withdrawal). At 48h uterine weights were measured (percentage of total body weight) and mRNA was subjected to qRT-PCR for E responsive genes lactoferrin and progesterone receptor (PR). Analysis of genes and uterine weight included an E-added group. Differences in endometrial restoration were assessed using a morphological scoring system[2].

Uterine weight was not different between control and E-free, but significantly higher with E-added (0.06±0.02% vs 0.08±0.01% vs 0.19±0.01%). Control lactoferrin mRNA (1.02±0.75 relative units) was not different to E-free (3.1±2.9), but E-added was significantly higher (176.8±190.6, $p \leq 0.05$). PR mRNA results were similar (6.8±5.9 vs 6.0±3.6 vs 87.2±60.7 relative units, $p \leq 0.05$). Thus extra-ovarian estrogenic influences are minimal after ovariectomy. Importantly, scoring of uterine morphology showed complete endometrial restoration in all groups within 48h of P withdrawal.

In conclusion, E is not required for successful endometrial restoration in this model, suggesting it also may not be essential for re-establishment of endometrium in women.

(1) Brasted et al. (2003) Biol Reprod 69:1273-80
(2) Kaitu'u-Lino et al. (2007) Cell Tiss Res 328(1): 197-206
(3) Britt et al. (2005) Menopause 12:174-85

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UTERINE GENE EXPRESSION DIFFERENCES BETWEEN SUPERIOR AND INFERIOR RECIPIENT COWS.

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Early embryo loss accounts for 22% of pregnancy failure in cattle. Previous work identifying recipient cows that had high or low holding rates, revealed no overt differences between ovarian factors [1] but a recent trial examining protein profiles in the uterine luminal fluid (ULF) suggested that the uterine response to pregnancy in these cows was dissimilar.[2]

After an oxytocin challenge thirty-five cows were selected as superior recipient (SR n=17) or inferior recipient (IR n=18) and were then synchronised for estrus followed by transfer of a grade one *in vitro* produced blastocyst on Day 7.

Fifty-five percent IR cows were non-pregnant 11 days after transfer or had a conceptus length <10cm whereas 76% of SR cows had a conceptus >10cm. The ULF was examined by Western blotting for the amount of IFN- t, UCRP and TIMP-2. Total IFN- t was less in the IR compared to SR cows. This difference resulted in lower concentrations of total UCRP in IR than SR uterine tracts. In the cows with conceptuses >24cm in length (n=15), there was significantly less (P=0.05) TIMP-2 in the gravid compared to the non-gravid ULF's of SR cows whereas IR cows had similar levels of TIMP-2 in both horns.

There was no significant difference in mean endometrial gene expression of osteopontin, IGFBP-2, IGFBP-3, glycam, UTMP, CSF-1, Cox-2, VEGF, erythropoietin, PPAR g and oxytocin R in SR compared to IR cows when matched for conceptus size. In pregnancies that had a conceptus >24cm, TIMP-2 mRNA mean levels were 1.5 times higher in IR than SR gravid endometrial tissue (P=0.01) whereas DKK-1, frizzled-4, and angiogenin were higher in SR than IR (1.5, 1.4 and 3, times respectively, $P < 0.02$).

Selection of cows with a high pregnancy holding ability that have early differences in their uterine environment in response to pregnancy has been confirmed by this trial.

(1) Peterson & Lee, 2003. Theriogenology 59:686-697
(2) Ledgard et al. 2006. Reprod.Dom.Anim. V1:547

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LYSOPHOSPHOLIPIDS MAY INFLUENCE THE ONSET OF LABOUR BY INCREASING EXPRESSION OF CONTRACTILE ASSOCIATED PROTEINS IN HUMAN MYOMETRIUM

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The onset of human labour is determined by changes in uterine contractility, receptivity and local concentrations of uterotonins such as prostaglandins (PGs) and oxytocin (OT). We have proposed that the threshold for labour may be lowered by circulating lysophospholipids (LPLs) such as lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P). These LPLs are liberated, in part, through the action of a placental-derived enzyme, lysophospholipase-D on substrates lysophosphatidylcholine (LPC) and sphingosylphosphorylcholine (SPC). To test the hypothesis that LPLs promote labour-associated changes in myometrial gene expression, we employed the PHM-1 pregnant myometrial cell line to investigate the effects of LPLs on contractile-associated protein (CAP) expression and myosin light chain (MLC) phosphorylation *in vitro* by immunoblotting. PHM-1 cells were shown to express S1P and LPA receptors by RT-PCR. LPA, LPC and SPC (5 μM) all increased the expression of connexin-43 gap junction protein after 8 and 16 h treatment relative to controls, with similar efficacy to PGF2a and OT (100 nM). S1P exerted only modest effects that did not reach significance. Immunocytochemistry revealed enhanced staining of anti-connexin-43-positive granules in the cell cytoplasm, consistent with enhanced abundance of connexin-43 in the Golgi. S1P and SPC increased COX-2 protein expression ~2-fold, similar to PGF2a , while LPA, LPC and OT had no effect. Expression of the pro-contractile EP1 receptor was increased (2-3-fold) by SPC, LPA, LPA and PGF2a . All agents tested altered the expression of progesterone receptors (PR), but had no consistent effects on the ratio of PRA, PRB or PRC, despite some apparently dramatic changes seen at individual time points. MLC phosphorylation (at 20 min) was significantly increased by SPC and LPA, with LPC and OT exerting a small, non-significant effect. These results support the proposition that LPLs promote pro-contractile changes in the myometrium and hence are regulators of the timing of labour and delivery at term and preterm.

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PROTEOMIC IDENTIFICATION OF PROPROTEIN CONVERTASE 6 SUBSTRATES IN DECIDUALISED HUMAN ENDOMETRIAL STROMAL CELLS

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Determination of the molecular components involved in normal human endometrial function is essential to our understanding of the regulators of implantation.

We previously identified the serine protease, proprotein convertase 6 (PC6) as a crucial mediator of decidualisation and successful implantation. Administration of PC6 antisense morpholino oligonucleotides greatly impaired decidualisation of human endometrial stromal cells (HESCs) (1) and blocked implantation in the mouse uterus (2). Since the action of PC6 is to regulate the activity of other proteins by post-translational cleavage or limited proteolysis, we aimed to identify downstream substrates of PC6 in HESCs as additional proteins involved in the preparation for successful implantation.

HESCs were isolated from endometrial tissue, grown in culture for one passage before being treated with the decidual stimulus, 8-bromo-cyclic-AMP for 3 days to induce decidualisation. Cell lysates were collected and the precipitated protein treated with or without recombinant PC6 (rPC6) for 2 hours in the presence of the PC fluorogenic substrate pERTKR-AMC to monitor rPC6 cleavage efficiency. The resultant protein products were CyDye labelled, isoelectric focused across a pI range of 3-10 and analysed by 2D Differential in Gel Electrophoresis (2D DiGE) to identify changes in the protein profile. 16 protein products were upregulated upon rPC6 addition while 11 proteins were more abundant in the control samples lacking rPC6. Many of the proteins upregulated in rPC6 treated lysates have low molecular weights and high pIs consistent with potential cleavage products. All of these products are currently being analysed by mass spectrophotometry for identification. It is anticipated that one or more of these products may be useful as future targets for contraceptive technology.

(1) Okada, H., et al., 2005. J Clin Endo Metab. 90(2): 1028-34.
(2) Nie, G., et al., 2005. Biol Reprod. 72: 1029-36.

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MICRORNA EXPRESSION IN ENDOMETRIOSIS

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Endometriosis causes pain and subfertility in 10-15% of reproductive aged women. mRNA microarray analysis has identified many genes that are differentially expressed in paired eutopic and ectopic endometrial samples. However, the mRNA levels of some proteins known to be elevated in endometriotic tissues were unchanged in these experiments, suggesting post transcriptional regulation. MicroRNAs are short single stranded, non-coding RNAs that can repress protein expression post transcriptionally. We hypothesised that microRNAs are differentially expressed in endometriotic lesions when compared to eutopic endometrium. Ectopic and eutopic endometrial samples were obtained from 7 naturally cycling women with AFS stage II-IV endometriosis. RNA extracted from these paired samples was fluorescently labelled and co-hybridized to microarrays containing 377 Ambion microRNA capture probes.The microRNA array identified 28 differentially regulated microRNAs, 18 up regulated and 10 down regulated. Several microRNAs were from the same microRNA gene family clusters and some microRNAs targeted transcripts known to be important in the endometriotic disease process. The differential expression of six microRNAs was confirmed using quantitative RT-PCR We have demonstrated for the first time differential expression of microRNAs in endometriotic tissues when compared to eutopic endometrium. These non-coding RNAs are likely to contribute to the pathophysiology of endometriotic lesion development and could be a target for pharmacological intervention in this debilitating disease.

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MIFEPRISTONE TREATMENT HALTS IMPLANON-ASSOCIATED BREAKTHROUGH BLEEDING BY ENHANCING ENDOMETRIAL REPAIR.

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Many women using progestagen (P)-only contraceptives experience irregular and/or prolonged episodes of uterine bleeding. In clinical trials, a single low dose of mifepristone, (progesterone antagonist), given to Implanon users at the beginning of a bleeding episode reduced the number of bleeding days by ~50% vs. controls[1]. In this study we used a mouse model for long-term P exposure and breakthrough bleeding[2] to elucidate the underlying mechanisms of this successful mifepristone treatment.

Pseudopregnant mice received a decidual stimulus to one uterine horn and a subcutaneous etonogestrel implant on day 4 of pseudopregnancy, designated 0d; complete endometrial decidualisation occurred by 2d[3]. A single dose of mifepristone (200µg in arachis oil) was administered subcutaneously at 5d and mice were culled 12, 18, 24 and 48 hours post-treatment (n=5). Control mice received vehicle alone (n=5). Morphological changes were assessed with emphasis on tissue breakdown and repair.

Mifepristone caused rapid tissue breakdown with rapid re-epithelialization around the basal zone of the decidua. By 48hr post-treatment (7d) most treated tissues were fully restored to the pre-decidualised state. Control tissues showed some tissue breakdown characteristic of this time point, but no evidence of epithelial repair.

Proliferating cells (Ki67 immunostained) were largely localized to a band of cells around the basal area of breaking-down tissue. In repairing tissue, positive cells were localized to the regenerating luminal and glandular epithelium.

Progesterone receptor positive (PR+) cells were largely localized to the basal area of the breaking-down tissue in treated mice, compared to decidual cells in controls. In repairing tissue after mifepristone treatment, PR+ staining was observed in some sub-epithelial stromal cells with little staining in other cellular compartments.

These studies suggest that bleeding in the presence of Implanon, occurs due to tissue fragility, including loss of epithelial integrity. Mifepristone treatment, in the continued presence of etonogestrel probably causes further instability in the tissue, sufficient to induce rapid restoration of the epithelium and hence reducing bleeding time.

(1) Weisberg et al. Hum. Reprod. 2006; 21:295-302
(2) Morison et al. Reprod. 2007; 133:309-321
(3) Brasted et al. Biol. Reprod. 2003; 69:1273-80

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A SWITCH FROM PARACELLULAR TO TRANSCELLULAR FLUID TRANSPORT MECHANISMS IN RAT UTERINE EPITHELIAL CELLS AT THE TIME OF IMPLANTATION

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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 under investigation.

Fluid can be transported across epithelia in two ways, between the cells (paracellular) and through the cells (transcellular). The paracellular pathway is regulated by tight junctions while transcellular fluid movement occurs through the membrane itself, either by diffusion or specific transporters such as aquaporins which are transcellular water channels.

This study investigated the expression of tight junction molecules occludin and claudin 4 as well as aquaporin 5 in rat uterine epithelial cells during early pregnancy using immunofluorescence, western blot and real time PCR. An upregulation of these molecules were all found in uterine epithelial cells at the time of implantation suggesting an increase in the tightness of the tight junction and thus a decrease in paracellular transport at the time of implantation. The upregulation of aquaporin 5 channels at this time suggests that fluid is removed via transcellular means. The expression of these molecules could explain the loss of luminal fluid at the time of implantation and suggests a mechanism for regulation of luminal fluid dynamics.

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BMP-2 AND TGFB-1 INCREASE DURING HUMAN ENDOMETRIAL STROMAL CELL DECIDUALIZATION

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Blastocyst implantation is dependent on the differentiation or decidualization of human endometrial stromal cells (HESC) into decidual cells. Transforming growth factor family members have well defined roles in cell differentiation and proliferation. Within the endometrium, activin A localises to decidual cells and accelerates decidualization in vitro. Similarly, recent knock out studies in mice have demonstrated that BMP-2 is critical for decidualization.[1] To date, many other TGFβ superfamily members have not been identified in the human endometrium. The aim of this study was identify specific BMPs (-2, -4 and -7) and TGFβ-1 in HESC in vitro and in vivo, and to assess secretion levels during decidualization. We hypothesized that BMP and TGFβ1 secretion would increase during HESC decidualization. HESC were decidualized in vitro using cAMP (0.5mM) for four days. RT-PCR was carried out for gene expression studies and culture medium collected for ligand specific ELISAs (n=3 cultures). Immunohistochemical analysis for BMP-2, -4, -7 and TGFβ-1 were performed on mid-late secretory human endometrium and first trimester placenta. BMP-2, -4 and -7 and TGFβ1 expression was identified in decidualized HESC. BMP-2 secretion significantly increased (17.6 ± 1.2 pg/10^6cells vs 9 ± 1.0 pg/10^6cells) (mean ± sem), as did TGFβ-1 (20.6 ± 3.8 pg/10^6cells vs 14 ± 3.1 pg/10^6cells) during HESC decidualization in vitro. However, BMP-4 secretion did not change and BMP-7 was undetectable. Immunoreactive BMP2 and TGFβ1 were both identified in decidualized cells of human endometrium during the mid-late secretory phase and in first trimester decidua. BMP-4 and -7 were also identified within human endometrial stromal cells, however were not exclusive to decidualized stromal cells. This is the first study to identify and localize BMP-2, -4 and -7 in human endometrium and early pregnancy. This data suggests BMP-2 and TGFβ-1 may play a role during decidualization, whilst BMP-4 and -7 may be involved in other endometrial processes. Elucidation of factors involved during decidualization will aid in better understanding implantation and fertility.

(1) 1 Lee KY, Jeong JW, Wang J, Ma L, Martin JF, Tsai SY, Lydon JP, Demayo FJ. Mol Cell Biol. Epub ahead of print, 2007.

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APICAL DISTRIBUTION OF EZRIN IN ALL RAT UTERINE EPITHELIAL CELLS AT THE TIME OF IMPLANTATION EXCEPT THOSE WITHIN THE IMPLANTATION CHAMBER

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Uterine epithelial cells undergo a 'plasma membrane transformation' that is a unique transition from 'typical' epithelial cells with regular microvilli to become 'blastocyst receptive' cells that have lost their terminal web and microvilli and have an increased number of intramembranous proteins in their apical membrane. This study investigates the role that ezrin, a cytoskeletal-membrane linking protein plays in the remodeling of uterine epithelial cells during early pregnancy. Ezrin displays highly specific distributions seen using immunofluorescence and increases in activity at the time of implantation as seen using protein fractionation and western blotting.

Ezrin distributes apically in any epithelial cells in contact apically with opposing epithelial cells and is therefore upregulated apically upon lumen closure at the time of implantation. Upon insertion of an inanimate siliconised filament into the uterine lumen, epithelial cells contacting the filament also displayed an apical distribution of ezrin. This finding determines that the upregulation of ezrin at the apical membrane does not differentiate between cellular and non-cellular contact. Ezrin migrates apically in uterine epithelial cells at the time of implantation in all uterine epithelial cells except in the unique circumstance of cells in direct contact with the blastocyst.

This suggests that it functions to link actin filaments below the apical membrane to adhesion molecules within the apical membrane as a means to organize these molecules in preparation for blastocyst adhesion to the uterine epithelial cells, but is then immediately dissociated from the membrane upon blastocyst contact. Ezrin dissociation upon blastocyst contact could indicate the early stages of apoptosis of the epithelial cells within the implantation chamber. The differential expression and increase in ezrin activity at the time of implantation suggests that ezrin plays an important role in organization of the apical membrane in preparation for implantation of the blastocyst.

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OESTROGEN HAS DIFFERENTIAL EFFECTS ON VEGF-A ISOFORM AND RECEPTOR MRNA EXPRESSION IN DIFFERENT CELLULAR COMPARTMENTS OF THE MOUSE UTERUS

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Recent research suggests that oestrogen (E) can be either pro- or anti-angiogenic in the endometrium. We have shown that E stimulates endometrial angiogenesis (endothelial cell proliferation) in ovariectomised mice, but also downregulates VEGF-A isoform and receptor mRNA expression in whole uterine tissue. This suggests that E initially stimulates angiogenesis, but also acts to prevent excessive angiogenesis. An alternative explanation is that VEGF-A is downregulated specifically in the endometrial epithelium, which has the highest VEGF-A expression and is not thought to have a role in endometrial angiogenesis, or that VEGF-A expression in the myometrium overshadows expression within the endometrium. We have examined the effects of E on VEGF-A isoform expression in specific compartments of the mouse uterus [epithelium, stroma (fibroblast, leucocytes and endothelial cells inclusive) and myometrium]. Ovariectomised mice (n=7) were treated with a single injection of E (100 ng) or vehicle (V) and dissected 24 hours later. Uterine epithelial, stromal and myometrial cells were collected using laser capture microscopy and mRNA expression of the VEGF-A isoforms ($VEGF_{120}$, $VEGF_{164}$, $VEGF_{188}$) and receptors (VEGFR-2, Nrp-1) were quantified by real time RT-PCR and normalized against 18S rRNA. E stimulated a significant decrease in VEGF-A isoform and receptor mRNA expression in the endometrial stroma. VEGF-A isoform, but not receptor expression, was also reduced in the myometrium. Except for $VEGF_{188}$, which significantly increased, no change in VEGF isoform and receptor mRNA expression was observed in endometrial epithelium. Downregulation of VEGF-A and its receptors within the endometrial stroma identifies a potential mechanism for the anti-angiogenic effects observed in response to E. We have yet to elucidate how E interacts with VEGF-A to control the pro-angiogenic effects within the endometrium.

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OSTEOPOROSIS MANAGEMENT

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Osteoporosis is best regarded as an integral part of the aging process, rather than as a disease entity. The key initial step in the management of osteoporosis is not the diagnosis of the condition, but the prediction of fracture risk. This depends on clinical risk factors (particularly age, weight and past history of fractures) and the measurement of bone density. Bone density can be measured at a number of sites, but the spine and hip are the most commonly used . With increasing age, degenerative artefacts have a substantial impact on the value of spine density measurements, so the hip is usually the preferred site.

Interventions available for the reduction of fracture risk can be partitioned into pharmacological and non-pharmacological. In the first category are life-style changes intended to reverse risk factors (e.g. cessation of smoking, maintenance of adequate body weight, moderation of alcohol intake). Calcium supplementation has been shown to produce sustained reductions in bone turnover and in the rate of bone loss in normal postmenopausal women. Recently, we have observed upward trends in the incidence of cardiovascular

disease in trial subjects randomised to calcium. If confirmed, this finding suggests that calcium supplementation is unlikely to have a net benefit. Vitamin D deficiency is common in the elderly and probably leads to accelerated bone loss. Some trials of combined calcium and vitamin D supplementation indicate a reduction of fractures in the frail elderly, but this has not been a universal finding. Other non-pharmacological means for reducing fracture risk include falls prevention programmes and the use of hip protectors.

The mainstay of pharmacological therapy is the bisphosphonates. These drugs adhere tightly to mineralised surfaces and are then ingested by osteoclasts during bone resorption. This results in loss of function and/or apoptosis of the osteoclast . They have a very long duration of action which favours their intermittent use. Typically , they are administered as a weekly or monthly oral dose, or as a 3 or 12 monthly intravenous dose. Bisphosphonates reduce vertebral fractures by 50 to 70%, hip fractures by 30 to 50%, and total fractures by 10 to 25%. Hormone replacement is a very effective therapy for osteoporosis, but concern about its other effects has greatly reduced its uptake. The SERMs have beneficial effects on bone density and vertebral fracture risk, but to date have been less effective than bisphosphonates. Parathyroid hormone is a peptide given by daily injection which promotes bone growth. Its costs, and concerns about long term safety, have limited its use to date.

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THE ROLE OF GHRELIN AND ITS CNS TARGETS IN THE CONTROL OF ENERGY METABOLISM

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Abstract not provided

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AMYLINOMIMETICS FOR METABOLIC DISEASES

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Amylin is released with insulin from pancreatic β-cells in response to nutrients and other insulinogenic stimuli. Amylin's most potent actions include slowing of gastric emptying, suppression of post-prandial glucagon secretion, reduction of food intake, and inhibition of secretion of acid and digestive enzymes from the stomach and exocrine pancreas respectively. These actions point to a physiologic function of amylin to regulate the rate at which nutrients are assimilated and released into the circulation. Effects of replacement therapy in patients with absolute amylin deficiency (type 1 diabetes) or relative amylin deficiency (late type 2 diabetes) using the human amylin analog, pramlintide, include improved glycemic control and reduced body weight, consistent with such a physiologic function.

Several, if not all, of amylin's actions appear to be centrally mediated. Focal lesioning studies indicate that effects upon gastric emptying and food intake at least, involve the area postrema, a circumventricular organ devoid of a blood-brain-barrier that has access to circulating peptides. Amylin binds with picomolar affinity to heterodimeric CTR+RAMP1 amylin receptors in area postrema and other circumventricular organs. Some actions (slowing of gastric emptying) require an intact vagus nerve, while others (post-prandial glucagonostatic effect, effect on food intake) do not. Several amylinergic actions (slowing of gastric emptying, inhibition of nutrient-stimulated glucagon secretion, inhibition of gastric acid secretion) are glucose-dependent, in that they are over-ridden during hypoglycemia. The glucose-dependence of some amylin actions may be explained by the finding that the activity of almost all amylin-sensitive neurones in single-unit extracellular recordings of area postrema were glucose-sensitive, showing inhibition by the presence of a low ambient glucose concentration.

In summary, via several parallel centrally-mediated glucose-dependent actions, amylin physiologically regulates the rate of entry of nutrient into the circulation. Its function may thus be viewed as complementing that of insulin (secreted from the same pancreatic β-cells), which controls the exit of nutrient from blood and promotes nutrient storage in peripheral tissues.

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NEW TRICKS FOR AN OLD GUT PEPTIDE: CHOLECYSTOKININ AND SYMPATHETIC OUTFLOW TO THE SPLANCHNIC CIRCULATION

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Cholecystokinin (CCK) is released from gastrointestinal tract enteroendocrine cells upon consumption of food to modulate several gastrointestinal functions including satiety. Our laboratory has demonstrated that CCK influences sympathetic vasomotor drive to the gastrointestinal circulation via an intramedullary trisynaptic pathway that includes activation of a vagal input to neurons in the solitary tract nucleus of the dorsal medulla and a relay to GABAergic inhibitory neurons in the caudal ventrolateral medulla. These, in turn, inhibit presympathetic vasomotor neurons in the rostral ventrolateral medulla (RVLM) resulting in splanchnic sympathoinhibition. CCK selectively inhibits the discharge of a sub-population of these RVLM presympathetic, spinally-projecting, vasomotor neurons. They are predominantly non-catecholaminergic, receive a dense input from serotonin-containing terminals, and all express cocaine and amphetamine-regulated transcript. CCK inhibits splanchnic sympathetic vasomotor outflow by activation of CCK1 receptors expressed on sub-diaphragmatic vagal sensory afferents. This pathway may be an important regulator of the gastrointestinal circulation and may be involved in post-prandial gastrointestinal hyperaemia and post-prandial hypotension.

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THE ROLE OF PYY IN REGULATING ENERGY BALANCE AND GLUCOSE HOMEOSTASIS

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The gut hormone peptide YY (PYY) belongs to the neuropeptide Y (NPY) family along with pancreatic polypeptide (PP). PYY is mainly produced by the endocrine L cells of the lower gastrointestinal tract as well as in the alpha cells in the pancreas, in the stomach and in the brainstem. There are two endogenous forms of PYY: PYY1-36 and PYY3-36. The latter is produced by the action of the cell surface enzyme dipeptidyl peptidase IV and is more selective for the Y2 receptors. Upon ingestion of a meal, PYY levels rise within 15 minutes, peak at 60 minutes and remain elevated for up to 6 hours in humans. Both PYY1-36 and PYY3-36 suppress appetite and food intake in rodents and humans and obese people exhibit reduced circulating PYY levels, but it is unclear whether this is a consequence or cause of obesity. We therefore investigated the effect of PYY ablation and PYY over expression on energy homeostasis. PYY knockout significantly increases body weight and fat mass on a normal diet and become significantly fatter and glucose intolerant compared to wild types when fed a high-fat diet. PYY knockout animals exhibit significantly elevated fasting or glucose-stimulated serum insulin concentrations versus wild types, with no increase in basal- or fasting-induced food intake. PYY knockout exhibited significantly increased growth hormone releasing hormone expression in the ventromedial hypothalamus and significantly elevated serum IGF-1 and testosterone levels. Interestingly, PYY transgenic mice are protected against diet-induced obesity in association with increased body temperature (indicative of increased thermogenesis) and sustained expression of thyrotropin-releasing hormone in the paraventricular nucleus of the hypothalamus. Moreover, PYY transgenic mice crossed onto the genetically obese ob/ob background had significantly decreased weight gain and adiposity, reduced serum triglyceride levels and improved glucose tolerance compared to ob/ob controls. Together, these findings suggest that long-term administration of PYY, PYY-like compounds or agents that stimulate PYY synthesis in vivo can reduce excess adiposity and improve glucose tolerance independently of effects on food intake, possibly via effects on the hypothalamo-pituitary-thyroid axis and thermogenesis. On the other hand reduced PYY levels may predispose to the development of obesity, particularly with ageing or under conditions of high fat feeding.

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GENE TESTING – ITS ROLE IN ENDOCRINOLOGY

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The molecular basis of many endocrine disorders is now known. Our laboratory has a strong interest in the pathogenesis of endocrine tumour syndromes which began with the identification of the RET oncogene as being pathogenic in Multiple Endocrine Neoplasia type 2. Since then the genes that are mutated in von Hippel Lindau disease (VHL), hyperparathyroidism – jaw – tumour syndrome (HRPT2), familial paraganglioma/phaeochromocytoma (succinate dehydrogenase – SDHX genes), Cowden's syndrome (PTEN), familial hypocalciuric hypercalcemia, (Calcium-sensing receptor – CaR) have all been identified.

Gene testing for these disorders is undertaken in families with these disorders or in individuals with apparently sporadic presentation to either include or exclude these diagnoses. Testing is performed on DNA prepared from peripheral blood and genes are amplified using polymerase chain reaction. Amplified sequences are then either screened using denaturing gradient high performance liquid chromatography (dHPLC) and/or directly sequenced. Consent is specifically obtained for genetic testing and two tubes of blood obtained on all individuals.

The identification of carriers of mutation in a disease gene either confirms a diagnosis or identifies an individual to be at risk of developing particular problems. Mutation positive individuals can be targeted for intense biochemical and radiological screening, and in the case of MEN2, prophylactic thyroidectomy has been shown to be effective in reducing morbidity and mortality.

Some of the important issues surrounding the genetic testing in the above conditions will be covered in this session.

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NATRIURETIC PEPTIDES AND ADJUSTMENT OF THERAPIES IN CARDIAC FAILURE

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Plasma BNP and NT-proBNP are powerful markers of symptom status, severity of cardiac dysfunction and prognosis. Serial testing could potentially be used to monitor clinical status and guide treatment in chronic heart failure. Plasma BNP and NT-proBNP levels at a single time-point in stable outpatient settings provide independent prediction of mortality and new heart failure events across all stages of heart failure. Serial testing provides incremental prognostic information, with a fall in levels at follow-up predicting fewer heart failure hospitalizations or deaths and a rise predicting a greater likelihood of these adverse outcomes.

In clinically stable individuals, variation in plasma levels between serial tests reflects analytical accuracy and altered secretion and clearance due to normal and pathophysiological processes, including myocardial ischemia, renal dysfunction and neurohormonal activation. Clinically undetected elevation of cardiac filling pressures may contribute to variability as may alternative peptide splicing. Variability is lower when peptide levels are high and above target levels in intervention studies. Long term intra-individual variation in NT-proBNP levels is in the order of 30%, with a change of more than 23% likely to indicate a change beyond background variation. When log-transformed peptide levels are assessed, variability is less than 10%.

NT-proBNP levels change with diuretic and vasodilator therapy, while withdrawal of diuretics is associated with a rise in peptide levels. Introduction of b -blocker therapy in stable mild heart failure is associated with an initial rise in NT-proBNP levels that is not due to clinical decompensation. Longer-term, levels fall, paralleling changes in LV remodeling. NT-proBNP levels also fall with cardiac resynchronisation therapy reflecting improvements in LV volumes and ejection fraction.

Because lower levels of NT-proBNP are associated with better clinical outcome, titration of treatment to achieve lower NT-proBNP levels may be advantageous compared to standard empiric therapy. Vasodilator therapy can be titrated to achieve lower natriuretic peptide levels. In two recent randomised studies, treatment targeted to achieve lower BNP or NT-proBNP levels resulted in fewer combined heart failure decompensation, hospitalisation and mortality events when compared to clinically guided treatment.

This strategy is being tested currently in several larger randomised trials in large cohorts that are representative of real-life heart failure populations with normal or reduced (<40%) ejection fraction and receiving modern therapy.

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SALIVARY STEROIDS

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The analysis of steroids in saliva dates back several decades and since that time its popularity has increased due to the attractiveness of non-invasive, repeated and simple stress free sampling, as well as the notion that the salivary steroid level may correlate with the free steroid in circulation. It is now a popular sampling fluid for psychobiology, sports medicine, pharmacology and paediatric studies as well as in the area of complimentary medicine. In the diagnostic laboratory saliva progesterone and oestradiol have been used for assessing ovarian function and 17OH progesterone for the diagnosis of congenital adrenal hyperplasia. The determination of androgens in saliva is more problematic and saliva testosterone shows variable correlation with circulating free testosterone, especially in females. The most promising application is the measurement of saliva cortisol. It is now used for investigating adrenal function, in part due to reports that plasma free cortisol is more robust than total plasma cortisol in assessing adrenal sufficiency. Recently there has been considerable interest in the use of bedtime saliva cortisol levels as a screening test for Cushing's disease and repeated sampling has proved helpful in the diagnosis of cyclical Cushing's disease. However an element of caution is required on the use of saliva which include collection techniques, the variable matrix of saliva, sensitivity, steroid stability and the presence of binding proteins. There is also the need for method specific reference ranges as methodological, standardisation and specificity issues can arise unless analysis is carried out by reference methods.

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INSULIN-LIKE GROWTH FACTORS AND THEIR BINDING PROTEINS IN ENDOCRINOLOGY AND CANCER

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The insulin-like growth factors, IGF-I and IGF-II, are peptides of approximately 7.5 kDa which are abundant in the circulation and the cellular environment, where they exert anabolic and mitogenic actions, and also stimulate cell survival, differentiation and motility. The liver is a major site of IGF production, though recent data suggest that adipose tissue also makes a significant contribution. Serum IGF-I, which is GH dependent, is commonly measured to monitor GH secretory status or action, whereas measurement of IGF-II, which is GH-independent, is mainly used to diagnose and monitor IGF-II secreting tumours, which typically manifest as hypoglycemia of non-pancreatic origin. The IGFs mainly circulate bound to six binding proteins (IGFBPs), of which IGFBP-3 is predominant in adults. Ternary complexes containing IGF-I or IGF-II, IGFBP-3 or IGFBP-5, and ALS (acid-labile subunit) circulate at approximately 100 nmol/L, providing a large IGF reservoir which, if fully bioavailable, would have profound metabolic effects. Regulation of its bioavailability is at least partly mediated by proteases which can lower the IGF-IGFBP affinity. At the cellular level, IGFBPs regulate the access of IGFs to their receptors, either inhibiting or potentiating IGF actions depending on the cell context. IGFBPs also affect cell function through mechanisms independent of IGF binding, transmodulating both cell-surface receptors such as the EGF receptor, and nuclear receptors including the retinoic acid and vitamin D receptors. Measurement of serum IGFBP levels can provide information about GH responsiveness (IGFBP-3), hepatic insulin sensitivity (IGFBP-1) and PI 3-kinase pathway activity in certain tumours (IGFBP-2), while ALS measurement is also useful as a marker of GH action, perhaps more liver-specific than IGF-I. Recombinant IGF-I is approved as a treatment for growth disorders characterised by IGF-I deficiency or GH insensitivity, and is the only effective treatment for children with growth deficiency due to GH receptor mutations.

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ESTROGEN DENDRIMER CONJUGATES ACTIVATE NONGENOMIC PATHWAYS OF ESTROGEN ACTION IN PROSTATE CELLS

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Estrogens, in combination with androgens, play an essential role in the development in prostate disease in aging men. Activation of estrogen signalling via the estrogen receptor subtypes (ERα or ERβ) regulates prostate cell growth and differentiation and selective estrogen receptor modulators (SERMS) are being investigated for the treatment of prostate cancer. However estrogens also stimulate nongenomic signalling pathways through rapid activation of membrane initiated kinase cascades, as recently described in breast cancer cells. The aim of this study was to examine if there is any evidence that non-genomic pathway activation occurs in prostate cells and if so, whether the activity varies between normal and malignant cells.

Initial results using an estrogen dendrimer conjugate (EDC), that does not directly activate estrogen responsive genes, show that ERK is phosphorylated in prostate cancer (PC3) cells in a time and dose dependent manner with maximum response observed within 10 to 20 minutes. In contrast to PC3 cells, the non-malignant prostate (PNT1A) cells show no increased levels of phosphorylated ERK, although constitutive levels are higher in these cells than PC3 cancer cells.

These data provide evidence of nongenomic rapid signalling of estrogen in prostate cells that may also contribute to abnormal cellular responses leading to prostate disease; further studies are underway to elucidate the downstream effects.

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MATERNAL OBESITY IN THE RAT LEADS TO INCREASED BODY WEIGHT, ADIPOSITY, INSULIN AND IMPAIRED GLUCOSE TOLERANCE IN OFFSPRING

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Animal models are a useful tool for understanding the physiology of appetite regulation and obesity. In rodents consumption of a palatable, high fat diet (HFD) results in a doubling of caloric intake, leading to increases in body weight, adiposity, plasma leptin and insulin concentrations. Maternal obesity is a known risk factor for increased body weight in offspring. An inevitable consequence of our rising rates of obesity is increasing numbers of obese mothers, increasing the risk of childhood obesity. Female Sprague Dawley rats were rendered obese by allowing access to unlimited palatable HFD. Chow fed females served as control. After 6 weeks they were mated with chow-fed males, continuing on their pre-mating diet. Rats were distributed at PD1 into litters of 3 (small) or 12 (control). Body weight, glucose tolerance, plasma insulin, adiposity and organ size of male offspring was recorded. At weaning body weight and fat mass was significantly increased in rats from obese mothers, and this was exaggerated in those from small litters. Glucose tolerance was impaired in the obese small litter group, with significantly increased AUC. Insulin was doubled in rats from obese mothers relative to those eating chow.

	Lean Control	Lean Small	Obese Control	Obese Small
Weight (g)	33.1 + 0.2	47.4 + 1.9 *	47.4 + 1.4 †	61.3 + 2.5 * †
WAT (%BW)	0.08 + 0.01	0.17 + 0.02 *	0.17 + 0.02 *	0.48 + 0.03 * †

* litter effect, † maternal obesity effect; n=12 $P < 0.05$, 2 way ANOVA

Significant litter effects were seen across both diets, supporting an important role of nutrition during the suckling period, which includes the critical window where hypothalamic appetite regulating projections are developing. The doubling of adiposity and insulin in offspring of obese mothers demonstrate the extent of the impact of maternal obesity. An understanding of the long term effects of maternal obesity is needed.

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ANDROGEN RESPONSIVENESS OF ANTERIOR AND DORSOLATERAL PROSTATE IN PROSTATE EPITHELIAL-SPECIFIC ANDROGEN RECEPTOR KNOCKOUT (PEARKO) MICE

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The cellular basis underlying androgen action in the mature prostate remain poorly understood. Using 8 week old PEARKO mice with androgen receptor (AR) inactivated selectively in the prostate epithelium (Simanainen et al., Endocrinology 148, 2007), we analyzed anterior (AP) and dorsolateral prostate (DLP) weight and epithelial volume in response to 2 weeks of orchidectomy and 1 week of treatment with 1 cm subdermal silatic implants of T or DHT.

In controls, T fully restored AP and DLP weights after orchidectomy whereas DHT was less effective. In contrast, full AP or DLP weights were not restored by androgens in PEARKO. Orchidectomy reduced AP and DLP epithelial volumes (by stereology) in both PEARKO and control, suggesting that reduction in epithelial volume due to castration mainly depends on changes in stromal androgen signaling. Yet, in PEARKO the androgen treatment produced incomplete restoration of epithelial volume in AP and DLP compared with controls which were fully rectified, indicating that epithelial AR is required for full epithelial androgen dependent regrowth and differentiation in the mature prostate. These results demonstrate the complex and distinct roles of androgen dependent

Experimental group		Lobe weight (mg)		Epithelial volume (% of total volume)	
		AP	DLP	AP	DLP
Intact	CTR	32 ± 2.0	15 ± 1.2	38 ± 3.2	33 ± 2.5
	PEARKO	9.8 ± 0.5*	6.7 ± 0.9*	53 ± 2.5*	45 ± 3.2*
Orchidectomy	CTR	6.6 ± 0.4$	4.0 ± 0.3$	29 ± 2.2$	20 ± 1.3$
	PEARKO	4.9 ± 0.7$	3.0 ± 0.3*,$	23 ± 2.0$	18 ± 0.4$
T	CTR	32 ± 2.2	13 ± 0.6	43 ± 0.7	32 ± 3.1
	PEARKO	7.3 ± 0.5*	4.5 ± 0.5*,$	37 ± 3.4$	25 ± 2.2$
DHT	CTR	24 ± 0.6$	11 ± 0.7$	43 ± 4.9	36 ± 2.1
	PEARKO	6.5 ± 0.4*,$	4.8 ± 0.4*,$	29 ± 2.3*,$	21 ± 2.4*,$

*=Significantly different (p<0.05) from littermate control (CTR)
$=Significantly different from respective intact

paracrine signaling between stroma and epithelia in maintaining prostate epithelial structure and function. Supported by Cancer Institute NSW, Cure Cancer, USYD Cancer Research Fund and Helsingin Sanomain Saatio.

(1) Simanainen et al., Endocrinology 148, 2007

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PLACENTAL INFLAMMATORY RESPONSE IN PREGNANCIES COMPLICATED BY ASTHMA

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Introduction: The placenta regulates fetal cortisol exposure during pregnancy. We have shown that cortisol bioavailability and regulation varies with gestation, fetal sex and the presence of a number of complications during pregnancy including preterm birth and asthma. Alterations in cortisol concentration may alter placental immune pathways particularly the balance between Th1 and Th2 cytokines. We examined the placental response to an immune challenge over time in the presence or absence of glucocorticoids of pregnancies complicated by asthma.

Methods: Placentae were collected from normal term deliveries, and term deliveries complicated by asthma (with and without maternal glucocorticoid treatment). Placental explants were cultured for 2 and 24hrs and then exposed to lipopolysaccharide (LPS), in the presence and absence of 100nM dexamethasone and 1 mM cortisol. At 2 and 24hrs supernatants were collected and assayed for TNF alpha by sandwich ELISA.

Results: At both 2 and 24hrs, the placental TNF alpha response to LPS was not significantly different between controls and asthmatics. At 2hrs, neither dexamethasone nor cortisol significantly inhibited the TNF alpha response to LPS in the asthmatics; however in controls, dexamethasone significantly inhibited the TNF alpha response to LPS. At 24hrs, dexamethasone and cortisol significantly inhibited the TNF alpha response in both controls and asthmatics. However, placentae from pregnancies complicated by asthma were less responsive to cortisol.

Conclusion: Placentae from pregnancies complicated by asthma may be less responsive to the effects of glucocorticoids. This may reflect a down-regulation of the glucocorticoid receptors in the asthmatic women allowing pro-inflammatory cytokines such as TNF alpha to predominate in the presence of an immune challenge.

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PLACENTAL AND FETAL GROWTH RESTRICTION REDUCE B-CELL MASS BEFORE AND AFTER BIRTH IN THE SHEEP

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Diabetes occurs when insulin secretion is inadequate to maintain insulin action for an individual's level of insulin sensitivity. Small size at birth in humans increases the risk of diabetes, and is associated with insulin resistance, with recent evidence for early onset impaired insulin secretion relative to sensitivity. Poor placental growth and function are major causes of poor growth before birth. We have shown that restricted placental and fetal growth (PR) in sheep impairs insulin secretion from early in postnatal life, with later onset insulin resistance in adult sheep (1-3). We have also shown that PR induces pancreatic expression of genes implicated in compensatory up-regulation of β-cell mass, in the young sheep (3), suggesting attempts to compensate for their impaired insulin secretion. We have therefore investigated the effects of PR on β-cell mass before and after birth into adulthood in the sheep.

In the fetus near term, (143 days gestation, term ~150 days), pancreas weight, β-cell mass and islet density correlated positively, and the number of β-cells per islet correlated negatively, with fetal size. Similarly, in adult sheep, the volume density of β-cells and β-cell mass correlated positively with size at birth, while islet density and the number of β-cells per islet were not related to size at birth.

Thus, placental restriction and poor growth before birth reduces β-cell mass, which persist into adult life and contributes to impaired insulin secretion. This suggests that the attempts at β-cell compensation previously reported in the young sheep after birth (3), are insufficient to restore β-cell mass and respond to the onset of insulin resistance. The factors by which placental restriction and fetal deprivation limit β-cell mass and islet formation before birth and their plasticity and compensation after birth remain to be determined.

(1) MJ De Blasio et al. 2007 Endocrinology 148: 1350-8.
(2) JA Owens et al. 2007 Am J Physiol 292: E1879-89.
(3) ML Harland et al. 2006 Proc ESA Endocrine Journal Suppl.: abstr. 126

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HUMAN *IN VITRO* FERTILISATION (IVF) DERIVED GRANULOSA CELLS: *IN VITRO* CHARACTERISATION AND COMPARISON OF TWO VIABILITY ASSAYS

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IVF-derived human granulosa cells (GC) are commonly utilised for physiological and toxicological studies. This study i) identified isolated GC using Oil Red O (ORO) lipid stain and 3β-hydroxysteroid dehydrogenase (3βHSD) activity, ii) optimised *in vitro* culture conditions with respect to cell viability, estradiol (E_2) and progesterone (P) production, and iii) compared two cell viability assays: the mitochondrial dehydrogenase based MTT assay and the DNA staining Crystal Violet (CrV) assay. GC were isolated from the follicular aspirates of IVF patients, using a continuous Lymphoprep^{TM} gradient (δ= 1.077g/L). Viable GC numbers were determined using Trypan Blue before culture in Chambertek II slides or 96 well plates. GC adherence was determined by seeding gradients of 0 to 4×10^4 GC/well in triplicate, and culturing for 2, 4 and 24h. Similar cell gradients were also cultured for 48, 72 & 96h and media assayed for P and E. After 48h 97.8% ± 4.0%, (n=3) GC were ORO-positive and 92.9% ± 12.4%, (n=3) GC were 3βHSD-positive. Optimal adherence time and seeding densities were 24h and 2×10^4 cells/well respectively; these conditions corresponded to maximum P production (1.9 ±1.6ug/mL), and E_2 concentrations of 0.0074± 0.004 μg/mL. The MTT assay generated higher viable cell numbers than the CrV assay, but both assays showed that cell numbers increased: wells seeded with 20000 GC contained 35000±19700 (n=4, MTT) or 32000±10300 (n=4,CrV) cells/well after 96h incubation, the latter corresponding to a 62% increase in viable cells. Continued luteinisation of GC in culture may have caused an increase in mitochondrial dehydrogenase activity, thus affecting MTT results. We therefore conclude that the CrV assay is more accurate and applicable for use with GC. If the 7% of cells that were non-steroidogenic doubled every 24hr, there would be a 62% increase in 96h, thus confirming previous reports that steroidogenic GC do not proliferate.

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MANHOOD THREATENED: COULD ESTROGENIC PESTICIDES AFFECT MALE FERTILITY?

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It has been suggested that exposure to agricultural insecticides may affect male fertility. Pyrethroids are widely used insecticides due to their insecticidal potency and low mammalian toxicity. We used a recombinant yeast assay system to analyze the estrogenicity of a range of readily available pyrethroid pesticides. The pyrethroid compounds bifenthrin, cypermethrin, and pyrethrum, and a synthetic metabolite of permethrin [3-(4-hydroxy-phenoxy) benzyl alcohol] tested positive for estrogenic activity based on ability to activate the α-estrogen receptor in the yeast assay. To determine whether these compounds could have an effect on male fertility mouse Sertoli cells were exposed to the endogenous estrogen, 17β-estradiol, and selected estrogenic pyrethroids. The effect of these compounds on gene expression of the α-and β-estrogen receptors was assessed at the levels of mRNA and protein. The effect on estrogen receptor levels of exposure of the Sertoli cells to the pyrethroid compounds, at both high and physiologically relevant concentrations, was different to the effect of exposure to 17β-estradiol. From these results we suggest that male fertility could be affected through molecular mechanisms involving the estrogen receptors, should cells in the male testes be exposed to pyrethroids at physiologically relevant concentrations.

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INVESTIGATION INTO THE EFFECT OF CREAP ON CRH PROMOTER ACTIVITY IN JEG-3 CELLS

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The cAMP regulatory element (CRE) is one of the most important elements controlling corticotropin releasing hormone (CRH) in the placenta and hypothalamus. Previously a placental cDNA library was screened using the yeast one-hybrid system to identify proteins capable of binding the CRE. A human cDNA for CRE Associated Protein 1 (CREAP1) was discovered. This novel protein has a distinctive combination of modular domains including two leucine-zipper-like domains, two zinc finger-like domains, two coiled-coil domains and an SR-rich domain characteristic of proteins involved in RNA splicing.

Pilot data from transfection experiments in JEG-3 cells, suggests that CREAP has little effect on CRH promoter activity, compared to the CRE binding protein, CREB. JEG-3 cells were transfected with a 663bp section of the CRH promoter, linked to luciferase. Co-transfections were performed with full-length and truncated CREAP, empty expression vector (PCIneo) and CREB. Cells were treated for 24hrs with vehicle, 0.1uM dexamethasone, 1uM phorbol ester (PMA) or 0.5mM 8-Br-cAMP and assayed for luciferase activity. As expected, CREB co-transfection resulted in increased CRH promoter activity under all treatment conditions. However CREAP co-transfection generally showed only modest changes in CRH promoter activity in vehicle, dexamethasone and PMA

treated cells and even tended to have an inhibitory effect on promoter activity in cAMP treated cells. Endogenous levels are quite high, so overexpression may not have much effect.

Small interefering RNA has been used to knockdown CREAP protein expression in JEG-3 cells. Around 80% knockdown of protein levels can be achieved without causing cell death. Microarrays and other methods are being used to determine the effect of overexpression and knockdown of this novel multifunctional protein.

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HERITABILITY OF SERUM TSH, FREE T4 AND FREE T3 CONCENTRATIONS: A STUDY OF A LARGE UK TWIN COHORT

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Thyroid hormone action influences many metabolic and synthetic processes, but the degree of regulation attributed to genetic influences, or heritability, and the influence of environmental factors on normal variation remain controversial. Using structural equation modelling to estimate variance components in the context of a classical twin study, we evaluated the genetic and environmental contributions to serum levels of free T3, free T4, TSH and the thyrotroph T4 resistance index (defined as the fT4*TSH product).

A large cohort of female twins, comprising 849 dizygous and 213 monozygous twin pairs (mean age 45, range 18–80 years) from the St Thomas UK Adult Twin Registry formed the basis of the study.

A comparison of thyroid parameters within various groups showed no differences between smoking categories (current, ex- or never smokers). Serum TSH was higher and free T3 lower in subjects with positive thyroid antibodies.

After adjusting for relevant covariates (age, smoking, antibodies, body mass index), the heritable contribution to serum thyroid parameters (with 95% confidence intervals) was 65% (58-71%) for TSH, 65% (58-71%) for thyrotroph T4 resistance index, 39% (20-55%) for free T4 and 23% (3-41%) for free T3. For free T3, unique environmental effects exert the greatest influence in this twin group, at 43% (35-52%).

Our analysis shows that genetic factors play a major role in determining the variation of serum thyroid parameters in healthy Caucasian women. These data have important implications for our understanding of pituitary-thyroid axis set points and the associations between thyroid hormone parameters and phenotypes such as body mass index, blood pressure and atrial fibrillation, which have been reported in population-based studies.

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FIRST TRIMESTER SPECIFIC REFERENCE INTERVALS FOR THYROID HORMONES

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Introduction: Maternal thyroid dysfunction during pregnancy has adverse effects on foetal development and is associated with increased risk of miscarriage. Currently, thyroid function during pregnancy is assessed using reference ranges derived from non-pregnant subjects. However the applicability of 'population' reference intervals is questionable, since in pregnancy the stimulating effect of beta-HCG on thyroid hormone secretion results in reduced serum thyrotropin concentrations.

The aim of this study was to establish first trimester specific reference ranges for fT4, fT3 and TSH to assist in identifying pregnant women with thyroid dysfunction , in whom treatment may be warranted.

Method: Free thyroxine (fT4), free tri-iodothyronine fT3), thyrotropin (TSH), thyroid peroxidase (TPO) antibodies and thyroglobulin antibodies (ATG) were measured on stored serum obtained from women attending Western Diagnostic Pathology for first trimester screening during October to November 2006. Samples were analysed by chemiluminescent immunoassays on the Abbott Architect.

Results: Of the 2159 samples assayed, 250 patients (11.6%) were excluded due to TPO and/or ATG positivity. The 2.5th and 97.5th percentile reference intervals were determined by non-parametric analysis. Results were as follows:

Gestation Weeks	n	TSH mU/L	fT4 pmol/L	fT3 pmol/L
9.0 – 9.6	104	0.02 – 2.36	11 - 17	3.3 – 5.4
10.0 – 10.6	712	0.02 – 2.13	10 - 18	3.3 – 5.6
11.0 – 11.6	544	0.02 – 2.22	10 – 18	3.3 – 5.6
12.0 – 12.6	427	0.08 – 2.00	10 – 17	3.3 – 5.9
13.0 – 13.6	118	0.04 – 2.46	10 – 17	3.2 – 6.0
ALL	***1905***	***0.02 – 2.17***	***10 – 18***	***3.3 – 5.7***
Non Pregnant Adult		**0.4 – 4.0**	**9 – 19**	**3.5 – 5.5**

By including the antibody positive patients, TSH increased by 0.4-0.7 mU/L at the high end.

Conclusion: The reference range for TSH in first trimester pregnancy should be lower than that of the general population. Application of the TSH reference range obtained from this study to women in the first trimester of pregnancy would enable identification of women with thyroid dysfunction, and in particular a group with subclinical hypothyroidism who would not be recognised in current practice.

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TRIMESTER-SPECIFIC THYROID FUNCTION TEST REFERENCE RANGES FOR IODINE SUFFICIENT PREGNANT WOMEN ATTENDING AN AMBULATORY ANTENATAL CLINIC

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Pregnancy is associated with significant physiological changes that affect maternal thyroid function and iodine status. Thyroid and iodine adequacy are essential for the health of both mother and developing foetus, particularly in the first trimester.

For the first time, we studied an Australian antenatal cohort with the aim of defining normative data for trimester-specific thyroid function tests in relation to maternal iodine status. Knowing trimester-specific intervals facilitates early detection of maternal thyroid dysfunction, enables prompt initiation or adjustment of thyroid hormone replacement, and serves to minimise adverse foetomaternal outcomes related to thyroid dysfunction.

Free thyroid hormone levels (fT4 and fT3), thyroid stimulating hormone (TSH) and spot urinary iodine concentrations (UIC) were simultaneously obtained in each trimester. Subjects were excluded from this analysis if they (i) had a personal history of thyroid disease; (ii) were found to have positive thyroid auto antibodies (thyroid peroxidise or thyroglobulin); (iii) were discovered to be iodine insufficient (UIC <100 ug/L[1]) on the initial testing. A standardised questionnaire was used to ascertain exogenous iodine exposure at the time of enrolment.

To date, only 36% (n=60/167) of subjects were iodine sufficient. Table 1 shows trimester-specific data for iodine sufficient women for TSH, fT4, fT3 (median and reference range) and median UIC and also details the prevalence of iodine deficiency for the entire cohort.

TABLE 1	Trimester 1	Trimester 2	Trimester 3
TSH (mIU/L) *	1.08 (0.44-2.72)	1.19 (0.63-2.9)	1.08 (0.47-2.64)
fT4 (pmol/L) *	12.45 (11.4-15.2)	12.2 (8.7-16.5)	11 (8.1-13)
fT3 (pmol/L) *	4.4 (3.6-5.7)	4.5 (3.8-5.1)	4.3 (4-5.3)
Median UIC (ug/L)	161	171	131
UIC<50 ug/L	27%	29%	20%

* median and 2.5th-97.5th percentile range for each analyte

The preliminary results suggest that reference ranges for antenatal thyroid function tests are lower than in the non-pregnant state. Despite current recommendations [2] only 50% of the cohort was on appropriate iodine supplementation.

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NUCLEAR AND NUCLEOLAR LOCALISATION OF PARAFIBROMIN, THE PUTATIVE TUMOUR SUPPRESSOR ASSOCIATED WITH HYPERPARATHYROIDISM JAW TUMOUR SYNDROME AND SPORADIC PARATHYROID CARCINOMA

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Loss of function mutations in the putative tumour suppressor *HRPT2* encoding parafibromin have been identified in patients with Hyperparathyroidism Jaw Tumour syndrome (HPT-JT) and in the majority of sporadic parathyroid tumours. Complete absence of staining for parafibromin has diagnostic value for the identification of parathyroid carcinomas and HPT-JT related tumours. As a member of the PafI complex, parafibromin binds to RNA polymerase II and has a role in regulating a number of transcription-related events. Consistent with this role, we have previously identified a functional bipartite nuclear localisation signal (NLS) at residues 125-139 of parafibromin. We now show that parafibromin fused to enhanced green fluorescent protein (EGFP) also exhibits sub-nuclear localisation in HEK293 cells and co-localisation with nucleolin confirmed this to be nucleolar. Inspection of the parafibromin sequence revealed three putative nucleolar localisation signals (NoLSs) rich in the basic amino acids arginine and lysine at residues 76-92, 192-194 and 393-409. These putative NoLSs were able to mediate nuclear localisation of EGFP to a much lesser extent than the bipartite NLS. In contrast, the three putative NoLSs, but not the bipartite NLS, were capable or localising EGFP to the nucleolus.

Additionally, mutation of each NoLS, either alone or in combination in full length parafibromin fused to EGFP decreased the percentage of cells showing nucleolar localisation and increased nucleolar exclusion relative to wild-type parafibromin-EGFP. Therefore parafibromin contains a bipartite NLS that is the primary determinant of nuclear localisation and three NoLSs that play a minor role in nuclear localisation but are critical for the nucleolar localisation of this protein. Other tumour suppressors such as Rb, p53 and ARF can also localise to the nucleolus with roles in ribosomal RNA synthesis and processing, cell cycle progression and/or cell growth. Studies are currently underway to investigate the function of nucleolar parafibromin.

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RHTSH (THYROGEN®) IN THYROID CANCER FOLLOW UP: EXPERIENCE AT A SINGLE INSTITUTION

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rhTSH-stimulated ^{131}I-whole body scanning and serum thyroglobulin (Tg) measurement has been reported to allow the rapid, safe and effective follow up of patients with thyroid cancer without the debilitating symptoms of thyroxine withdrawal and with equivalent prognostic information to that obtained after thyroid hormone withdrawal. We report the results of rhTSH use at the Alfred Hospital, Melbourne, from 1999-2006. In Australia, CMBS-funded use is restricted to patients with specific documented psychiatric or cardiac conditions. Method: rhTSH was used in 90 patients for one or more of these criteria: 1. CMBS-funded criteria (n=10); 2. severe hypothyroid symptoms on previous withdrawal scanning (n=8); 3. Presumed low or no tumour burden, with previous negative withdrawal scan(s) (n=33), with no past negative withdrawal scan (n=30), or other (n=15); 4. Facilitation of ^{131}I therapy (100mCi) (n=18). We conducted retrospective chart review including histology, rhTSH-stimulated Tg (ICMA, Immulite 2000), and ^{131}I scan results. rhTSH (0.9mg IM) was given on days 1 and 2, with Tg sampling on days 1, 2, 3, and 5. ^{131}I (2mCi) was given on day 3. Whole body scanning performed on day 5. Scans were called negative in the absence of pathological uptake and Tg was called negative if it remained undetectable (< 1 mg/L) after rhTSH. Results: *Patients*: Aged 5-75 years old at diagnosis (n=35 >45 y), 70F, 20M. Known cancer duration was 9m to 32y, with 43 followed up for 5-15y and 47 for < 5y. *Histology*: papillary (PTC) n = 54, follicular variant PTC n = 16, follicular (FTC) n = 17, Hurthle cell carcinoma n = 3. *Staging*: (AJCC TNM 2002) 26 stage 1, 16 stage 2, 23 stage 3, 20 stage 4, and 5 unclassifiable. *rhTSH*: used for 96 diagnostic scans and 18 ^{131}I therapy doses. TSH maximum post-rhTSH mean =110.9+4.4 mU/L (n=85), median 99.3 mU/L (range 33.9 – 226.8). Only occasional mild headache or mild injection site discomfort seen. *Stage 1*: Scan and Tg results were congruent negative in 23. In 3, scans were negative but Tg detectable, highest 16 mg/L; *Stage 2*: 16/16 had congruent negative scans and Tg; *Stage 3*: 19 had congruent negative scans and Tg; 3 had negative scanning but detectable post-rhTSH Tg <15 mg/L consistent with low-volume disease on US or CT; 1/16 had chest uptake (no lesion on CT) with Tg 8.8mg/L. *Stage 4*: 2 had pathological uptake with Tg >300 mg/L; 7 had congruent negative scan and Tg; 9 had no pathological uptake with detectable Tg >1-50 mg/L including one with Tg >300 mg/L, with disease identified on US, CT or PET in 6 patients where these modalities were used; 2 with Tg < 10 mg/L had no further imaging. Conclusions: 1. rhTSH was well tolerated; 2. In no case did rhTSH-stimulated ^{131}I scanning identify the presence of disease not also identified by raised Tg; 3. When present, pathological ^{131}I uptake localised disease; 4. rhTSH-stimulated Tg identified presence of disease not seen on ^{131}I scanning but found on other imaging; 5. rhTSH can facilitate ^{131}I therapy.

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SIGNIFICANT ASSOCIATION BETWEEN SERUM FREE THYROXINE CONCENTRATION AND BODY MASS INDEX IN EUTHYROID SUBJECTS: A COMMUNITY-BASED STUDY.

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Background. Thyroid dysfunction has well-established effects on body composition, but there are conflicting data as to whether small differences in thyroid function within the reference range are associated with differences in body mass index (BMI). Furthermore, smoking status is associated with differences in BMI, but it is uncertain whether smoking modifies the relationship between thyroid function and BMI.

Aim. The aim of this study was to examine the relationships between thyroid function, BMI and smoking status in euthyroid subjects.

Methods. We examined associations between each of free T4 and TSH thyroid function and BMI in a community-based sample of 1853 euthyroid subjects with no history of thyroid disease and serum TSH between 0.4 and 4.0 mU/L (the Busselton Thyroid Study).

Results. There was a significant negative correlation between free T4 and BMI (r=-0.143, P<0.001); this relationship remained statistically significant after adjustment for age and sex. The mean BMI of subjects in the highest quintile of fT4 concentration was 24.4 ± 3.5 kg/m^2, compared with 26.1 ± 3.8 kg/m^2 for the lowest quintile.

When analysed by smoking status, BMI was significantly higher in ex-smokers than current and never-smokers. The relationship between FT4 and BMI that was seen in the whole cohort, was maintained in ex-smokers and never smokers, but not in current smokers.

There was no significant correlation between TSH and BMI (r=0.005, P=0.84) in the whole cohort, or in subgroups according to smoking status.

Conclusions. Small differences in free T4 are associated with differences in BMI which may be important at the population level. This relationship is lost in current smokers.

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THYROTOXICOSIS DURING SUNITINIB TREATMENT FOR RENAL CELL CARCINOMA

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Sunitinib malate is an oral tyrosine kinase inhibitor approved for use of the treatment of renal cell carcinoma and gastrointestinal stromal tumors. Hypothyroidism develops in more than 30% of patients treated with sunitinib[1], but the mechanism whereby sunitinib induces hypothyroidism is unknown.

Here we report five patients who developed thyrotoxicosis while on sunitinib for metastatic renal cell carcinoma[2,3]. Two patients developed severe thyrotoxicosis within less than 10 weeks after commencing sunitinib, one of whom died within days of presentation with multi-organ failure. In contrast, in the three patients who presented with later onset (16-34 weeks) thyrotoxicosis, the thyrotoxicosis was relatively mild, self-limiting and rapidly progressed to hypothyroidism. These patients experienced recurrent episodes of thyrotoxicosis in temporal relation to their cyclical sunitinib treatment with intercurrent recovery while off sunitinib. One patient had cytological evidence of lymphocytic thyroiditis. All patients had normal thyroid function tests (TFTs) prior to commencing sunitinib. With the exception of a distant history of Graves' disease in one patient, none had features predisposing to thyroid disease or evidence of thyroid auto-immunity.

Sunitinib-induced hypothyroidism may be a consequence of preceding sunitinib-induced destructive thyroiditis with associated transient thyrotoxicosis. Whether thyroiditis occurs because of modulation of immune tolerance or via interaction with tyrosine kinase targets expressed on thyroid cells is unknown. In our series, time to onset of thyrotoxicosis was unpredictable, and severity ranged from mild to life threatening. As predictive factors are currently unknown, we suggest monitoring of thyroid function every two weeks in all patients commenced on sunitinib. Severe thyrotoxicosis requires aggressive treatment especially as patients with poor performance status due to underlying illness may rapidly decompensate. In milder forms of thyrotoxicosis, expectant management may be the preferred option, especially as such patients may develop hypothyroidism within weeks. Clinicians treating patients with sunitinib or other similar kinase inhibitors should to be alerted to thyroid dysfunction being a potential toxicity of these agents.

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(3) This protocol was approved by the Austin Health Human Research Ethics Committee and all patients provided written consent

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PREVALENCE OF SUBCLINICAL HYPOTHYROIDISM AND AUTOIMMUNE THYROIDITIS IN PREGNANCY

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Background: Prevalence of subclinical hypothyroidism among pregnant women is found to be 2.5%. Early diagnosis is crucial as both mother and child are affected. There is two- fold increase in miscarriage rate as well as increased incidence of other pregnancy complications like abruptio placenta , gestational hypertension, anemia , preterm labour and postpartum haemorrhage. Fetus is also affected with multitude of effects like disordered brain development, IUGR, fetal distress etc.., Thyroid autoimmunity in itself is a risk factor for pregnancy loss probably because it acts as a marker of more generalized activation of immune system or for an underlying autoimmune thyroid disease.Early therapy with levothyroxine is found to improve pregnancy outcome in both subclinical hypothyroidism and autoimmunethyroiditis.

Objectives: 1.To assess the prevalence of subclinical hypothyroidism in 500 pregnant women 2.To assess the prevalence of thyroid autoimmunity in pregnant women with euthyroid function

Materials and Methodology: 500 pregnant women from outpatient department of IOG Egmore and RSRM hospital were screened irrespective of gestational age.Exclusion criteria consist of patient already having thyroid disease or on thyroxine replacement therapy. Screening methods include questionnaire along with general physical examination and specific examination for thyroid dysfunction. Blood samples were drawn for freeT4, TSH and TPO antibodies.

Results: Out of 500 pregnant women screened 51[10.2%] were in I trimester,292[58.4%] in II trimester and 157[31.4%] in III trimester . 236[47.2%] were primi and 264[52.8%] multigravida . Prevalence of subclinical hypothyroidism was found in 14[2.8%] of whom 8[57.1%] were TPO positive and 6[42.9%] TPO negative . Of the remaining 486 with euthyroid function TPO positivity was found in 34[7%] women of whom 20 were in II trimester.

Conclusion: 1.Screening of pregnant women for subclinical hypothyroidism and thyroid autoimmunity is vital as it has long term effects on both mother and fetus. 2.Most of the women with subclinical hypothyroidism are asymptomatic or symptoms are ascribed to pregnancy itself.This limits the role of clinical examination indiagnosis of hypothyroidism in early pregnancy and hence the need for screening. 3.Early replacement with levothyroxine therapy is indicated in both subclinical hypothyroidism as well as thyroid autoimmunity in euthyroid state as it improves pregnancy outcome.

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NUCLEAR TRANSPORT AT THE ONSET OF MAMMALIAN GONAD SEXUAL DIFFERENTIATION: IDENTIFICATION OF CARGO FOR IMPORTIN ALPHA 2 IN THE FETAL TESTIS.

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Formation of a mature sperm requires regulated nucleocytoplasmic shuttling of transcription factors and nuclear proteins (*1*) . We have documented changing synthesis and subcellular localisation of importin nuclear transport protein family members during mammalian testis development and in spermatogenesis (*2-4*) . Because each importin exhibits selective cargo transport, we hypothesised that regulated transport of specific proteins into the nucleus by importins may control gonadal differentiation in mammals. The importin alpha 2 (IMPα2) mRNA level exhibits a gender-specific spike at embryonic day 12.5 (E12.5), coincident with testicular sexual differentiation. We created a cDNA library from mouse testes (Swiss strain; dissected free of mesonephoi) in the Gateway™ vector system. The library was used for a yeast-2-hybrid screen using full length IMPα2 as bait. 41,600 clones were screened, and 5 candidate cargoes were identified. One clone encoded 418 aa (80%) of paraspeckle protein 1 (PSPC1), a component of nuclear paraspeckles and androgen receptor coactivator (*5*) . Co-transfection of full length PSPC1 and IMPα2 (both full length and mutant {transport-deficient} forms) in mammalian COS7 cells validated the observed binding in yeast. The incidence of paraspeckle-containing cells was reduced by 44% after co-transfection with mutant versus full length IMPα2. Hence fully functional IMPα2 may mediate PSPC1 nuclear translocalisation, enabling its paraspeckle localisation. Given the known role of PSPC1 in androgen-responsiveness (*5*) , we examined PSPC1 expression throughout mouse testis development using immunohistochemistry. PSPC1 was detected in fetal germ cells in ovary and testis. After birth, PSPC1 was observed in spermatogonia at 0, 6, 10 and 15 days postpartum (dpp) and in spermatocytes and Sertoli cells from 10 and 15 dpp onwards, respectively. At 26 dpp and in adults, PSPC1 was absent from spermatogonia but detected in spermatocytes and round spermatids, matching IMPα2 expression (*3*) . The presence of PSPC1 in gonocytes suggests it may influence androgen action in these cells (*6*) , as described in adult Sertoli cells. This is the first study to identify a cargo for importin alpha2 in fetal testes and leads to new insights in PSPC1 biology.

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EVIDENCE FOR A GLUCOSE SENSING SYSTEM REGULATING PHYSIOLOGY OF THE PREIMPLANTATION MOUSE EMBRYO.

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We present evidence that glucose may, through the hexosamine biosynthetic pathway (HBP), play a novel signalling role in the differentiative events leading to blastocyst formation. Glucose flux via HBP produces uridine 5'-diphospho-N-acetylglucosamine (UDP-GlcNAc), the donor substrate for various glycosylation reactions. O-glycosylation with N-acetylglucosamine moieties (O-GlcNAcylation) at serine/threonine residues is a dynamic modification functionally reciprocal to serine/threonine phosphorylation and so influences protein activity and/or stability. The role of perturbed glucose flux through HBP/O-GlcNAcylation during preimplantation physiology was assessed following pharmacological and nutrient manipulations in culture media. Zygotes (16-18 h post hCG) were cultured in KSOM as control or modified KSOM (hypoglycemia, hyperglycemia or glucosamine supplementation without glucose) to disturb HBP flux. HBP flux was also perturbed with benzyl-2-acetamido-2-deoxy-alpha-d-galactopyranoside (BADGP) (to inhibit β-linked-O-N-acetyl glucosamine transferase, the enzyme responsible for GlcNAc transfer) or streptozotocin (STZ) (to inhibit N-acetyl glucosaminidase, the enzyme responsible for GlcNAc removal). O-GlcNAcylation levels were assessed by confocal immunofluorescence and western immunoblotting with antibodies specific for the O-GlcNAcylation moiety (mean nuclear grey-scales/band intensities, with subsequent ANOVA) and physiological outcomes were determined by blastocyst formation rates

and total cell number counts. Relative to control KSOM cultured embryos, hypoglycemia, glucosamine and STZ increased in nuclear O-GlcNAcylation by 70%, 79% and 89% respectively (ANOVA, $P<0.05$, n≥2 experiments, 5 embryos assayed/treatment/experiment) whilst BADGP reduced O-GlcNAcylation by 52% (ANOVA, $P<0.05$, n≥2 experiments, 5 embryos assayed/treatment/experiment). Western immunoblotting confirmed the global increases in O-GlcNAcylation induced by hypoglycemia and hyperglycemia (ANOVA, $P<0.05$, n≥2 experiments, 100 embryos/treatment/experiment). More importantly, all the treatments that perturbed O-GlcNAcylation reduced blastocyst formation (ANOVA, $P<0.05$, n≥3 experiments, 20 embryos/treatment/experiment) and further, hypoglycemia, hyperglycemia and glucosamine reduced morulae cell number by 40%, 39% and 55% respectively relative to KSOM (ANOVA, $P<0.05$, n≥2 experiments, 6 embryos/treatment/experiment). These data indicate that nutrient availability regulates key events of mouse development through the HBP and suggests that glucotoxicity to embryos is in part mediated by HBP and O-GlcNAcylation.

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PATERNAL POLYMORPHISMS ARE ASSOCIATED WITH PREGNANCIES COMPLICATED WITH UTEROPLACENTAL INSUFFICIENCY

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Poor placental development is associated with a variety of common, potentially life-threatening pregnancy complications (PCs), including preeclampsia (PE) and small for gestational age (SGA) babies. Paternity is a known risk factor for PCs, with the risk of developing PE doubled if the man has previously fathered a PE pregnancy in any woman [1] or was born to a PE pregnancy himself [2]. The placenta and fetus express a combination of maternal and paternal genes, thus paternal genotype contributes to placental gene expression. We hypothesize that polymorphisms in paternal genes involved in placental development determine pregnancy success and are associated with uteroplacental insufficiency (UPI). We aimed to examine the prevalence of single nucleotide polymorphism (SNP), in two Genes which result in altered bioactivity of their encoded proteins, in pregnant women and their partners. Blood was collected from women attending the Women's and Children's Hospital and the Lyell McEwin Health Service and their partners. DNA was extracted and genotyped by PCR followed by high resolution melt (HRM). Women were classified as control (n=37), UPI [n=40, comprising PE (n=16), SGA (n=14), PE+SGA (n=6), Gestational Hypertension and/or Placental Abruption (n=4)] after delivery. The frequency of Gene A allele-1 homozygosity in fathers was reduced by 73.5% (p=0.018) in UPI (n=40) and by 100% (p=0.006) in SGA (n=14) pregnancies compared to controls (n=37). The frequency of Gene B allele-2 homozygosity in fathers tended to be increased in PE+SGA pregnancies (p=0.064, n=6) compared to controls (n=34). No association was observed between maternal genotype and pregnancy outcome. We are currently determining the effect of genotype on placental gene expression and how this may affect pregnancy outcome. These data show that paternal genotype may play an important role in pregnancy success, consistent with paternity being a risk factor for pregnancy complications. Our results also suggest that the activities of protein A and/or B may be reduced in the placenta of pregnancies affected by UPI.

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HOMEOBOX GENE *HLX1* IS A MEDIATOR OF HGF-STIMULATED TROPHOBLAST MIGRATION

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Trophoblast cells play critical roles in normal placental development. Abnormal trophoblast cell proliferation, migration and invasion are associated with common pregnancy disorders such as fetal growth restriction and pre-eclampsia. Hepatocyte growth factor (HGF) induces proliferation and migration of epithelial cells. In the placenta, HGF is produced in the stromal core of the villi and activates trophoblast cell functions in a paracrine fashion. In previous studies, we showed that the homeobox transcription factor gene *HLX1* mediates trophoblast cell proliferation following CSF-1 growth factor stimulation (1). Here, we investigated whether *HLX1* mediates HGF-stimulated trophoblast migration, using the well-characterised human trophoblast cell line SGHPL-4. We showed by semi-quantitative reverse transcriptase PCR and scanning densitometry that *HLX1* mRNA levels significantly increased in a dose-dependent manner following HGF stimulation (e.g. 43.2 ± 2.5, with HGF (10ng/ml) vs. 18.4 ± 1.7 control, densitometric units, $p<0.001$, n=3). Trophoblast cell migration was assessed using Transwell® assays in which. trophoblast cells that migrated through a membrane filter were stained and densitometric quantitation was carried out using image analysis software (Axiovision). Cell migration significantly increased following HGF stimulation ($4.1 \times 10^5 \pm 350$ vs. $2.2 \times 10^5 \pm 240$ densitometric units, $p<0.05$, n=4) as expected (2). Reduction of *HLX1* mRNA/protein levels by siRNA treatment (1) resulted in a significant decrease in cell migration ($7.5 \times 10^4 \pm 120$ vs. $2.2 \times 10^5 \pm 240$ densitometric units, $p<0.05$, n=4). Furthermore, when *HLX1* expression was decreased by siRNA in the presence of HGF stimulation, migration remained significantly decreased ($1.5 \times 10^5 \pm 170$ vs. $2.2 \times 10^5 \pm 240$ densitometric units, $p<0.05$, n=4). Thus, we have provided evidence that *HLX1* may be an important mediator of HGF-stimulated trophoblast cell migration. The generality of these findings will be further confirmed in other trophoblast cell lines.

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STUDIES OF THE ROLE OF ABC TRANSPORTERS AND SPHINGOLIPIDS IN TROPHOBLAST DIFFERENTIATION

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Placental trophoblasts differentiate & fuse throughout pregnancy in a unique process to form a multinucleated cell membrane (syncytium). However, the mechanisms underlying this fusion process remain unclear, although apoptotic pathways appear to be involved. Preliminary studies demonstrated that the expression of ABC (ATP binding cassette) transporters, MDR1/ABCB1 and BCRP/ABCG2, change dramatically during trophoblast differentiation. ABC proteins have recently been shown to transport sphingolipids known to regulate cell viability, apoptosis and fusion. We hypothesized that ABC transporters, via their effects on efflux and distribution of apoptotic and mitogenic sphingolipids, serve a fundamental role in promoting trophoblast survival and differentiation. Cultured trophoblasts, isolated from term human placentas by trypsin digestion, were allowed to differentiate over 7 days in culture; hCG and syncytin expression peaked around days 2-3 of culture and media levels of hCG reaching maximal concentrations at 3 days. Morphologically, fusion commenced around day 2 of culture and was complete by day 4. Caspase-8 activity peaked around day 2 of culture and declined thereafter, but increased with syncytial ageing; caspase-3/7 activity showed a similar pattern . MDR1 and BCRP protein expression showed opposite changes during differentiation, with BCRP increasing just prior to differentiation and then declining, while MDR1 abundance was high initially but declined precipitously with differentiation (day 3). Changes in expression of acid sphingomyelinase and ceramidase *in vitro* suggested increased ceramide generation with differentiation and enhanced ceramide metabolism thereafter. Total cellular C16 ceramide levels declined prior to differentiation and increased thereafter, in accordance with differentiation and apoptosis. These results are consistent with the hypothesis that sphingolipids and ABC efflux proteins are involved in trophoblast differentiation and placental function. Drugs that interfere with ABC function may, therefore, also indirectly alter placental development.

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EMBRYO PROGRAMMING: THE INVOLVEMENT OF MITOCHONDRIA.

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Adult health and disease onset can be programmed by fetal adaptations in response to environmental conditions during pregnancy. Recently we have shown that a perturbed environment applied to the embryo during pre-implantation development, in particular the first cleavage division, can alter fetal growth outcomes, indicative of embryo programming. The mechanism for this permanent adaptation is currently unknown. This study investigates whether irreversible changes in embryo mitochondria are involved in the fetal growth alterations associated with our established models of pre-implantation stress exposure.

Zygotes from F1 hybrid mice (CBAB6F1) were cultured to the 2-cell stage (19h) in either control medium or in the presence of one of two model stress systems that we have determined result in perturbed fetal growth a) 300μM NH_4^+ or b) 2mM DMO (5,5-Dimethyl-2,4-oxazolidinedione) . For assessment of reversibility, embryos were returned to control culture conditions for a further 24h (to the 8-cell stage). Mitochondrial homeostasis was determined by assessing membrane potential (MMP), reactive oxygen species (ROS) production and ADP or ATP levels. Differences between treatments were assessed by generalised linear modelling followed by post-hoc test.

Culture with NH_4^+ or DMO significantly reduced MMP by 19% and 9% respectively ($P<0.01$) in the cortical region of the 2-cell when compared to control. This effect was not able to be reversed after embryos were returned to control culture conditions for another 24h. Similarly, 8-cell embryo ROS levels were increased by both NH_4^+ (117%; $P<0.05$) and DMO (86%; $P<0.05$) exposure, despite the stress being removed after 19h of culture. At the 2-cell stage, exposure to NH_4^+ significantly decreased ADP levels whilst exposure to DMO significantly reduced ATP levels ($P<0.05$).

This study shows that exposure to NH_4^+ and DMO results in altered mitochondrial homeostasis and energy production and these responses to stress were not reversible. These findings show for the first time that mitochondrial dysfunction is involved in the embryonic stress response and may contribute to programming fetal outcomes.

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PLACENTAL MEDIATORS OF PREGNANCY AND PARTURITION

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Uncovering the mechanisms responsible for determining the timing of labour and delivery in women has proved to be a surprisingly elusive and complicated endeavour. Despite extensive knowledge from animal models concerning the central role played by the placenta in mammalian parturition, uncertainties regarding the significance of the placenta in human parturition compromise our understanding of the unique intricacies of human pregnancy.

Traditionally, the Nancy Sirrett lecture is presented in the form of a review of a career from a senior member of the NZSE approaching retirement. Since retirement is, at this point in time, far from my thoughts and plans, in this presentation I will attempt to provide from my own perspective and experience some insight into current thinking on the role of the placenta and its various endocrine and paracrine mediators in human parturition, a field of research which has interested and challenged me for the past 20 years.

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ADIPONECTIN AND ITS RECEPTORS IN INSULIN RESISTANCE, DIABETES, AND METABOLIC SYNDROME, AND OBESITY.

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Adiponectin is a major insulin sensitizing hormone, which activates AMP kinase and PPARα pathways to facilitate glucose and lipid metabolism to increase insulin sensitivity. Decreased plasma adiponectin linked to obesity is causally involved in insulin resistance and metabolic syndrome in obesity. Moreover, decreased plasma adiponectin is causally involved in atherosclerosis. In fact, decreased plasma adiponectin levels have been shown to be associated with future development of diabetes and cardiovascular diseases in humans. These biological effects of adiponectin are mediated via adiponectin receptors, Adipo R1 and Adipo R2. Activation of AdipoR1 by adiponectin stimulates AMP kinase pathway and that of AdipoR2 by adiponectin stimulates PPARα pathway, thereby ameliorating insulin resistance. AdipoR1 and AdipoR2 are down-regulated in obesity, which is also casually involved in insulin resistance linked to obesity. PPARγ agonists upregulate plasma adiponectin levels, which contributes to amelioration of insulin resistance and atherosclerosis. On the other hand, PPARα agonists upregulate adiponectin receptors. Recently, adiponectin has been shown to stimulate AMPK activity in the arcuate hypothalamus and increase food intake via AdipoR1. Moreover, adiponectin decreases energy expenditure. Adiponectin appears to serve as a starvation gene. Thus adiponectin is upregulated according to decreased white adipose tissue mass, which then centrally facillitates energy storage and peopherally facillitates fatty acid combustion to generate energy for survival. In times of plenty, adiponectin is downregulated according to increased white adipose tissue mass, namely obesity, thereby causing insulin resistance, diabetes, and the metabolic syndrome. Thus, adiponectin and adiponectin receptors are crucially involved in the development of insulin resistance, diabetes, metabolic syndrome, and obesity and thus may serve as molecular targets of treatment and prevention strategy of these diseases.

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THE MURINE BETAGLYCAN GENE IS ESSENTIAL FOR NORMAL SEMINIFEROUS CORD FORMATION.

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Betaglycan is a co-receptor that binds both transforming growth factor-beta (TGF-β) and inhibin, acting as a modulator of the activities of multiple members of the TGF-β superfamily. We have characterized the gonadal expression pattern of the betaglycan gene and its protein during murine embryonic gonadal development. Betaglycan mRNA and protein were detected in the somatic cells within the interstitium of the fetal testis from 12.5 *dpc*-16.5 *dpc*. Immunofluorescence experiments showed colocalization of Betaglycan and Cyp11a (P450 side chain cleavage SCC) in the Leydig cells of the 14.5 *dpc* fetal testes. Histological examination of betaglycan null testis revealed a defect in seminiferous cord formation at 12.5-13.5 dpc in which the cords are poorly delineated from the surrounding interstitium. In addition, coelomic vessel development appeared slightly delayed in some betaglycan null testes. In contrast, betaglycan null ovary did not exhibit any clear structural differences compared to wildtype. In the null testis, a study of cell-type specific markers indicated that all the major cell populations were present in null testis but that laminin deposition was irregular and discontinuous around the developing seminiferous cords. Due to the prominent expression of betaglycan in foetal Leydig cells, we used real time PCR analysis to detect changes in the steroidogenic markers in the betaglycan knockout mouse model. The level of mRNA expression of *Cyp11a* was downregulated at 13.5 *dpc* in the betaglycan knockout embryos in comparison with that of the wildtype, immediately after sex determination. In addition, immunohistochemistry on Bouin's-fixed, paraffin-embedded tissue sections showed that Cyp11a protein expression was reduced in the fetal interstituim. Our data indicate that betaglycan is expressed in the fetal Leydig cells of the male gonads and suggest a possible role for betaglycan in Leydig cell differentiation.

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PRODUCTION OF REGULATORY CYTOKINES BY LYMPHOCYTE SUBSETS IN THE RAT TESTIS IS CONSISTENT WITH ITS STATUS AS AN IMMUNE PRIVILEGED SITE

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The testis is an immunologically privileged tissue. Spermatogenic cells are potentially immunogenic, but normally avoid provoking an immune response, and grafts survive for extended periods in the testis of experimental rodents. The rat testis has been most studied in this regard, and intratesticular leukocytes (macrophages and lymphocytes) are implicated in mechanisms that make the rat testis a successful site for tissue transplantation. However, the mechanisms contributing to immune privilege remain poorly defined. We have previously shown that the rat testis contains a significant population of lymphocytes consisting of NK cells, T cells, and cells expressing both NK and T cell surface markers (NKT) cells. These lymphocyte subsets are important regulators of antigen-specific immune responses, through production of the type 1 (pro-inflammatory) cytokine interferon-γ (IFN-g) and the type 2 (anti-inflammatory) cytokine interleukin-4 (IL-4), in other tissues, but their immunological functions within the testis have not been defined previously. Lymphocytes were isolated from adult rat testes using a non-enzymatic procedure and cultured under basal conditions or stimulated with phorbol myristate acetate and ionomycin for 48h to collect medium or for 4h in the presence of the protein transport inhibitor brefeldin A. Lymphocytes were permeabilized with the detergent saponin and stained with IL-4, IFN-g, and IL-10 antibodies. Results were analyzed by multi-colour FACS analysis using Summit software. Cytokines in the culture medium were measured by ELISAs. Rat testicular NK cells, T cells, and NKT cells produced IFN-g upon stimulation but surprisingly failed to produce IL-4, differing from circulating lymphocytes, which are strong producers of this cytokine. Crucially, testicular T cells and NKT cells were found to produce significant amounts of IL-10 under both stimulated and unstimulated conditions. The production of IFN-g by NKT cells has been shown to play a role in preventing graft-versus-host disease in bone marrow transplantation studies. The immunosuppressive cytokine IL-10 has been shown to play important roles in maintaining immune privilege in other organs. Therefore, the inducible production of IFN-g and constitutive production of IL-10 by testicular lymphocyte subsets is expected to contribute to the unique immune responsiveness in this organ resulting in immune privilege.

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SHBG AND MEGALIN EXPRESSION IN THE DEVELOPING REPRODUCTIVE TRACT OF A MARSUPIAL, THE TAMMAR WALLABY

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Androgens mediate differentiation of the urogenital sinus (UGS) to form a prostate. There is mounting evidence to show that sex hormone binding globulin (SHBG)-bound steroids may play a more active role in sexual differentiation than previously thought. Megalin is a cell surface receptor expressed on epithelia, which mediates endocytosis of a wide range of ligands including SHBG-bound sex steroids. Megalin knock-out mice have disrupted androgen and oestrogen signalling, resulting in cryptorchidism and failure of vaginal opening (Hammes *et al.* 2005) . This study cloned tammar *SHBG* and *MEGALIN* and using RT-PCR, examined gene expression in the liver and kidney and in the reproductive tissues such as ovary, testis and urogenital sinus of male and female tammars that ranged in age from day of birth to day 80 post partum.

Tammar *SHBG* was 77% homologous with human SHBG. When the tammar *SHBG* sequence was translated to a putative protein sequence, it shared even higher similarity (83%) with human SHBG protein. *SHBG* mRNA was present in the liver, testis, ovary and urogenital sinus of developing male and female tammar pouch young. Likewise, tammar megalin was 82% homologous with human megalin, and was highly similar (84%) when the putative protein sequence was compared to human megalin protein. *MEGALIN* transcripts were detected in the liver, kidney, ovary, testis and developing urogenital sinus of male and female tammars. The presence of SHBG and megalin in the UGS of the tammar during the period of prostatic differentiation suggests that SHBG and megalin, either separately or together, may play a role in sex steroid signalling development of the reproductive tract in this marsupial.

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THE EXPRESSION OF STEROID 5A-REDUCTASES IN REPRODUCTIVE TISSUES DURING VIRILISATION IN THE TAMMAR WALLABY, *MACROPUS EUGENII*.

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Androgens produced by the fetal testis are responsible for the formation of the epididymis and the seminal vesicle, the prostate, the phallus and the male urethra (1). Testosterone produced by the developing testis is converted to a more potent androgen, dihydrotestosterone (DHT) through a catalytic conversion involving the steroid enzymes, 5α-reductases type 1 and type 2 (SRD5α1 and SRD5α2). Deficiences or mutations in the genes that encode these two enzymes results in serious reproductve syndromes such as pseudohermaphroditism. The tammar wallaby is an ideal animal model for studies of the process of virilisation because the testes, prostate and phallus differentiate after birth at widely separate times, allowing investigation of the control of virilisation in each tissue separately. This study focuses on the role of 5α-reductases in this process. Partial sequences of *SRD5α1* and *SRD5α2* in the

tammar were obtained by PCR using primers based on *Monodelphis domestica* database sequences and by cloning fragments of predicted size. Both isoforms in the tammar shared a high amino acid sequence homology of 90% with the *M. domestica* sequences. *SRD5α1* had an 82% sequence homology with man and 76% with mouse, while *SRD5α2* was 80% and 78% homologous with human and mouse respectively. Both genes were expressed in the testes and ovaries, urogenital sinus/prostate and urogenital tubercle/phallus of both male and female pouch young collected between the day of birth and day 150 post partum. Quantitative real time PCR studies of each of the two isoforms of 5α-reductase confirmed that there are dynamic changes in expression during development that are consistent with an important role of 5α-reduced androgens in virilisation of this species. Differences in the pattern of expression of *SRD5α1* and *SRD5α2* between tissues and stages suggest differential roles for each of these enzymes during virilisation in the tammar.

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ACTIVIN-REGULATED GENES IN THE DEVELOPING MOUSE TESTIS

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Transforming Growth Factor-β (TGFβ) superfamily ligands influence male germ cell development and testis function. Our ongoing investigations of genetically modified mice with different bioactive levels of activin showed testicular germ cell numbers at birth are increased in the absence of activin [2], and our more recent evidence indicates this difference emerges between 13.5 and 15.5 days post coitum (dpc). Testes from *inhba* knockout (-/-; [1]), heterozygote (-/+) and wild type (+/+;WT) animals (containing 0, 1 and 2 copies respectively of the gene encoding the activin βA subunit) were used to assess the basis of this affect of activin on testicular development. Bouins fixed paraffin embedded testes sectioned at 4 µ m were used for immunohistochemical analyses with markers of proliferation (BrdU and PCNA) and apoptosis (TUNEL) on fetal and newborn testes. There was no evidence of different apoptosis rates across genotypes at 13.5 and 15.5 dpc; germ cell proliferation rates are currently being established. There was a striking reduction in Sertoli cell proliferation in testes from newborn *inhba* -/- mice compared with WT littermates, a pattern not observed at earlier ages. Thus both somatic and germ cells are affected by activin A in the fetus. To identify activin target genes, Affymetrix™ and quantitative real time PCR were performed on independent pools of testes from animals of each genotype at 13.5, 15.5 dpc and newborn. Notably, mRNAs encoding the cell cycle regulators cyclin D_2, p21 and p27 were differentially regulated by activin levels through fetal testis development. We are now pursuing an understanding of the specific cell types in which these changes occur to further ascertain the mechanisms by which regulated TGFβ superfamily signalling influences testis growth.

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SELECTION OF RAMS FOR TEMPERAMENT DOES NOT AFFECT THEIR REPRODUCTIVE CAPACITY

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Temperament is defined as the fearfulness or reactivity with which an animal responds to novel or challenging situations and is a highly repeatable and heritable trait (1). Temperament affects a number of production traits including body weight, wool growth and the reproductive capacity of merino flocks (2,1). A large proportion of the variation in reproductive capacity appears to be due to improved maternal behaviour and lamb survival of calm compared to nervous ewes (2). To investigate if there is a male component to the improved reproductive capacity of calm sheep, we compared the sexual behaviour and reproductive physiology of rams from two lines of sheep selected for calm and nervous temperament. Rams (calm; n=6, nervous; n=6) were tested for sexual behaviour with an oestrous ewe, and their trainability and behaviour during semen collection with an artificial vagina (calm; n=5 and nervous; n=6). Sperm quality (calm; n=8, nervous; n=9), testicle size and circulating LH and testosterone concentrations (calm; n=12, nervous; n=12) were also compared. Calm rams sniffed the ewe more often than nervous rams ($P<0.001$) whereas nervous rams more frequently pawed the ewe than calm rams ($P<0.001$). However, no differences were found in either the mating behaviour or fertility of calm and nervous rams ($P > 0.1$). Therefore, temperament does not appear to affect the reproductive capacity of merino rams. Differences in the reproductive capacity of merino flocks (2) seem to be derived from the effect of temperament on the sexual receptivity (3), maternal behaviour (1) and perhaps fertility of ewes.

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DEVELOPMENT OF TOTIPOTENT STEM CELL LINE AND THEIR CHARACTERIZATION

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Conservation and multiplication of rare mammals is possible by reproductive cloning.Number of embryos forms through this technology is less and on transfer to the recipient forms disorders like epigenetic modification, low conception rate, fibrous fetal membrane,abortions and large calf syndrome leading to dystocia.Blastomere cloning is an alternative but the number of cells is limited.Totipotent stem cell line developed form pre compact morula(8-16 cells) is a probable solution.The present study is therefore was undertaken to develop totipotent stem cell line from pre compact morula to be used for reproductive cloning,to multiply a specific animal to identical multiples,in goat model.The characterization of these cells was done using stem cell markers.Goat pre compact morulae were developed from slaughter house ovarian oocytes by IVFMC of 559 oocytes(Crozet et al.,1995).The presumptive zygotes were further cultured in mSOF for 44-48 hours,61 oocytes cleaved and at 72 hours 20 embryos developed to pre compact morula(Thakahashi and First,1992).These morulae were made zona free by pronase treatment.The blasomeres in groups were then cultured in CR11 media(First et al.,1994)on mytomycin C inactivated fetal fibroblast monolayer with LIF. After 30 days of culture few cells were isolated and fixed on slides.They were used for characterization,using alkaline phosphatase (ALP) and Stage Specific Embryonic Antigen-1(SSEA-1).Plutipotent stem cells isolated from early fetal tissues were used as control. The study shows that 80% of the blastomeres divided and formed colonies of totipotent stem cells.As colony grows,cells detached form the original colony and formed fresh colonies in the culture dish.The intensity of markers, ALP and SSEA-1 was much lower than that of Pluripotent stem cells.It may be concluded from this study that totipotent stem cells can be developed in culture and there is a requirement of developing markers for totipotent stem cells as pluripotent stem cell markers do not work properly on these cells.

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ROLE OF EPITHELIAL STEM/PROGENITOR CELLS IN ESTROGEN-INDUCED ENDOMETRIAL REGENERATION

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Rare candidate endometrial epithelial stem/progenitor cells have been identified in mouse endometrium as label retaining cells (LRC).[1] The LRC technique identifies adult stem cells on the basis of their relatively infrequent rate of cell division compared to more mature cells. Epithelial LRC are ERα negative and are located in the luminal epithelium.[1] The aim of this study was to examine the proliferation kinetics of endometrial epithelial LRC during estrogen-induced endometrial regeneration in ovariectomised prepubertal and cycling mice. Postnatal day 3 female mice received multiple injections of BrdU for 3 days followed by a chase of 4 (prepubertal) or 8 (cycling) weeks, then ovariectomised to regress the endometrium for 14 days, followed by one 17β-estradiol injection to stimulate endometrial regeneration, and uteri were harvested 0-120 hours later. Sections were double immunostained with BrdU and Ki-67 or phosphorylated-histone H3 (PH3) to detect proliferating and mitotic LRC, respectively. In prepubertal mice, epithelial LRC commenced proliferation 8 hours after estrogen and were the first cells to undergo mitosis. LRC numbers (3.3%) remained constant over 120 hours, while the percentage of mitotic ($PH3^+$) or proliferating ($Ki\text{-}67^+$) epithelial cells progressively increased, reaching a maximum at 24 and 48 hours, respectively. In contrast, in cycling mice there was a basal level of mitotic (1.2%) and proliferating (5.8%) epithelial cells at time 0, which rapidly increased within 2 hours of estrogen (2.4% $PH3^+$ and 31.7% $Ki\text{-}67^{+)}$, reaching maxima at 24 hours. LRC also commenced proliferating 2 hours after estrogen and entered mitosis at 8 hours. The percentage of LRC (1.2%) remained constant over 120 hours and there was one round of cell division. This study demonstrates that epithelial LRC function as stem/progenitor cells, initiating epithelial cell mitosis and playing a major role in the growth and development of prepubertal endometrium, but appear to have a minor role during endometrial regeneration in cycling mice. Alternatively, there maybe more epithelial stem/progenitor cells than LRC in mouse endometrium.

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IGF-I AND INSULIN ACTIVATE MITOGEN ACTIVATED PROTEIN KINASE VIA THE TYPE 1 IGF RECEPTOR IN MOUSE EMBRYONIC STEM CELLS

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Although insulin-like growth factor I (IGF-I) and insulin are important modulators of preimplantation embryonic physiology, the signalling pathways activated by these ligands during development remain to be elucidated. As a model of preimplantation embryos, pluripotent mouse embryonic stem cells were used to investigate which receptor mediated actions of physiological concentrations of IGF-I and insulin on growth measured by protein synthesis. Compared to a basal medium lacking serum, mouse embryonic stem cells showed a 25% increase in incorporation of 3H-leucine into protein in response to IGF-I or insulin with the former being 100

fold more potent, suggesting action via IGF1R. This was confirmed using an anti-IGF 1R blocking antibody (αIR3) and investigating receptor autophosphorylation and participation of the ERK1/2 mitogen activated protein kinase pathway with western blotting and immunoprecipitation. The results suggest that IGF-I and insulin modulate embryonic stem cell physiology through binding to the IGF-1R and subsequent activation of mitogen activated protein kinase pathway.

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ISOLATING AND GENOTYPING SPERMATOGONIAL STEM CELLS

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Spermatogenesis is the ongoing process of gamete production and is reliant on self-renewing spermatogonial stem cells (or SSCs). These SSCs represent a population of biotechnological interest as targets for animal transgenesis or fertility control. Currently the most efficient method for the enrichment of SSCs from a germ cell pool is based on the binding of the integrin α 6 β 1 on the stem cell/spermatogonial surface to a laminin extracellular matrix. Assessment of SSC purification is based on germ cell transplantation. This approach is cumbersome for high throughput screening for a very small component of the total population.

In order to generate a faster method of assessing SSC/spermatogonial populations we have screened α 6 β 1^+ cells for the expression of 12 genes including the differentiation marker *c-kit*, pluripotency markers *(nanog, oct-4, stella*) and genes postulated to feature only in SSCs (e.g. *PLZF*). We can now identify potential SSC on the basis of high (>3 fold increase in α 6 β 1^+ cells) expression of four genes and low (>3 fold increase in α 6 β 1^- cells) expression of three genes as well as the absence of expression of two genes that are limited to only α 6 β 1^- cells.

Recent work has demonstrated that germ cell differentiation is not irreversible and spermatogonia can revert to a SSC phenotype under appropriate circumstances, such as access to an empty stem cell niche as is present during transplantation. Therefore SSC number based on testes germ cell repopulation following transplantation is necessarily an over estimate. To improve the efficiency of specific SSC isolation we employed phage display library "panning" to characterise the surface of these cells. The epitope-binding peptides identified have been expressed as recombinant proteins. As a result of this approach we have five unique tools that target SSC/spermatogonial subpopulations. These allow us to identify SSC and differentiated germ cells and we are now in a position to genotype subpopulations of spermatogonia with a view to characterising the differentiation process and identifying cells capable of re-establishing the male germline.

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EMBRYONIC STEM CELLS: AN APPROACH TO GERMLINE GENESIS

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Current *in vitro* systems for producing mammalian germline cells from embryonic stem (ES) cells have met with some success. Both our own and other studies have shown that ES cells, when differentiated as aggregates (embryoid bodies: EBs), can spontaneously express markers of the germline, albeit at a very low rate. In addition, the few offspring produced in published studies exhibit abnormalities[1]. To increase the efficiency of *in vitro* germline genesis, our approach is to more closely recapitulate the *in vivo* microenvironment. The TGFbeta superfamily ligands bone morphogenetic protein (BMP) 2, 4 and 8b are critical drivers of germline specification[2], and in this study we examined their impact on EB lineage development. An ES cell line containing the Oct4 distal enhancer-promoter-GFP reporter[3] was used to analyse the response to these ligands, as Oct4 expression is maintained only in the germline following differentiation. ES cells were grown as EBs in hanging drop cultures for up to 10 days with the BMP ligands. An optimised level of BMP4 alone enhances the number of GFP positive cells in Day 10 EBs (~7-fold over untreated). BMP2 acts to a similar degree (~6-fold), and although BMP8b has no effect on its own, the combination of all three growth factors leads to an increase of approximately 9-fold. Parallel increases were measured by quantitative-PCR in a range of mRNAs encoding germ cell markers, including *stella*, *nanog* and *dazl*. Furthermore, although BMPs can drive other cell fate outcomes, in our system the expression of markers of most other lineages is not significantly affected. The isolation and subsequent differentiation of the GFP positive EB-derived cells as aggregates with foetal and post-natal gonadal cells is now underway to identify conditions that will direct these cells further in germ cell differentiation. Thus, by altering the differentiation microenvironment of ES cells to more closely reflect development *in vivo*, we can enhance germline specification *in vitro*.

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STRUCTURAL INSIGHTS INTO LIGAND-INDUCED ACTIVATION OF THE INSULIN RECEPTOR

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The insulin receptor and the IGF-1 receptor are homologous multidomain glycoproteins that bind insulin and IGF with differing specificity. The structure of the IR kinase domain was solved in 19941 but the structure of the extracellular ligand-binding domain has proved elusive until recently2 . Our structure is of the IR ectodomain dimer bound to Fabs from the monoclonal antibodies 83-7 and 83-14. This structure reveals an unexpected folded-over conformation for the IR which places the ligand-binding regions in juxtaposition. This arrangement is very different from previous models. It shows the two L1 domains to be on opposite sides of the dimer, too far apart to allow insulin to bind both L1 domains simultaneously. Instead, the structure implicates the loops at the junction of the the first and second fibronectin-III domains as the second binding site involved in high-affinity binding2 . The IR is heavily glycosylated and contains a total of 19 predicted N-linked glycosylation sites distributed across each of the constituent monomers. To provide a more complete description of the receptor structure. We have characterized the composition of the O-linked3 and most of the N-linked glycans unpublished and modeled the most prevalent glycoform at each site onto the IR crystal structure. We have also determined the structure of the first three domains of the IR at 2.2 Å resolution and compared it with the corresponding fragment of IGF-1R4 . There are major differences at the two regions governing ligand specificity. One difference is at the corner of the ligand-binding surface of the L1 domain where the orientation of the side chain of Phe39 is very different to that of its counter-part Ser35 in the IGF-1R. The second occurs in the 6th module of the cysteine-rich region, where IR contains a larger loop, negligible sequence identity, more a -helix, an additional disulphide bond and opposite electrostatic potential compared to IGF-1R4 . The implications of these findings for ligand-induced receptor activation will be discussed.

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CRITICAL NODES IN SIGNALLING PATHWAYS: INSIGHTS INTO INSULIN ACTION

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Abstract not provided

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DISSECTING MULTIPLE STEPS OF GLUT4 TRAFFICKING AND IDENTIFYING THE SITES OF INSULIN ACTION

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Abstract not provided

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NEW ROLES OF IRS-2 IN COMPENSATORY BETA CELL HYPERPLASIA AND VASCULAR FUNCTION

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Abstract not provided

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THE GONADAL HORMONE, MÜLLERIAN INHIBITING SUBSTANCE, HAS CRYPTIC FUNCTIONS IN THE CENTRAL NERVOUS SYSTEM.

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Functional MRI and epidemiological studies of psychiatric and neurological disorders suggest that sexual dimorphism occurs in many regions of the brain. Embryos are initially sexually indifferent, with the male phenotype being largely induced by androgens. This includes the male features of the brain. Virilisation, however, is also dependent on a gonadal protein which is referred to as either Müllerian Inhibitory Substance (MIS) or anti-Müllerian hormone. MIS is a member of the TGF-beta superfamily whose functions were thought to be limited to triggering the regression of the uterine anlagen in male embryos and to regulation of the mature gonads. We have recently discovered that motor neurons express receptors for MIS (MISRII and ALK3) and that MIS supported the survival of motor neurons in vitro (1). This suggests that MIS may virilize the motor nervous system, as MIS in the embryo is male-specific. We report here that the numbers of motor neuron in male $MIS^{-/-}$ and $MISRII^{-/-}$ mice have a feminine form. MIS receptors were also detected in various other regions of the brain using MISRII-reporter mice, real-time PCR and

immunohistochemistry. Most of the MISRII+ve neurons have no direct function in reproduction. This raises the issue of whether MIS has a broad role in creating gender-specific differences in the brain. In the adult, MIS ceases to be dimorphic and is produced by the gonads of both sexes, at least until menopause. We also report that motor and some other neurons begin to produce MIS once the brain has developed. Consequently, the brain appears to be bathed in a hormone and local regulator whose function is currently unknown.

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TGFβ SUPERFAMILY SIGNALING AND GONADAL FUNCTION

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It is now possible to modify the mouse genome to generate transgenic mice with precise genetic mutations, and thousands of such models have now been created. For the last 15 years, our laboratory has been using knockout and knockin transgenic mouse studies to define TGF beta superfamily signaling pathways and function. In our efforts to understand reproduction in vivo, we have produced transgenic knockout models for studying TGF? superfamily ligands (e.g., growth differentiation factor 9, bone morphogenetic protein 15, activins, and inhibins), a ligand binding protein (e.g., follistatin), a receptor (e.g., ACVR2), a putative downstream receptor binding protein (e.g., FKBP12), downstream SMADs, and a downstream target (e.g., pentraxin 3). These investigations have revealed that TGF-? superfamily ligands, receptors, SMADs, and upstream and downstream regulators function in diverse developmental and physiological pathways. We are extending our studies by using new technology such as Cre-loxP conditional knockout strategies to address the in vivo roles of these and additional proteins in mammalian reproduction. In particular, we have focused recent attention on the roles of all of the receptor-regulated SMAD proteins in gonadal physiology. These studies implicate multiple SMADs in post-natal gonadal function. Our research in this area have been supported by the National Institutes of Health.

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THE NEW ENDOCRINOLOGY OF ACTIVIN AND FOLLISTATIN: EXPLORING THEIR ROLES IN INFLAMMATION AND OTHER CRITICAL DISEASES

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Activin A and follistatin were originally described for their property in regulating release of follicle-stimulating hormone from the pituitary gland. Since then, further functions have been elucidated, including mesoderm induction in the early embryo, facilitating neuroprotection, during apoptosis and in other cellular and tissue processes. We have been investigating two in particular, that of inflammation and fibrosis, and exploring their relevance to human disease conditions. One of our original observations in this area highlighted a dramatic and rapid increase in circulating concentrations of activin A following systemic administration to sheep of the bacterial cell wall component, lipopolysaccharide (LPS) . Activin increased within 40 minutes in the bloodstream and preceded by some minutes that of the early pro-inflammatory cytokine, tumour necrosis factor-α. An elevation in circulating concentrations following inflammatory stimulation was confirmed in the clinical setting of septicemia, where both activin A and follistatin were increased in patients with this condition and where levels of activin tended to indicate patient outcome. We have since confirmed in a mouse model of lethal endotoxemia that circulating activin concentrations correlate with survival or mortality. Other clinical inflammatory conditions where we have shown activin is elevated are in meningitis, traumatic brain injury and burn injury. In terms of fibrotic processes, we have shown that activin A is involved in the activation of fibroblast-like cells and deposition of extracellular matrix. Use of follistatin to block activin effects in a rat model of liver fibrosis significantly attenuated development of hepatic fibrosis and activation of stellate cells. Follistatin has also been efficacious in a mouse model of allergic asthma by reducing structural damage and tissue inflammation. Overall, we have established activin A and follistatin as important new factors in inflammatory and fibrotic processes, with significant potential in developing diagnostic and therapeutic approaches in human disease.

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THE ESSENTIAL ROLES OF TGFβ1 IN REPRODUCTIVE BIOLOGY

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The reproductive tract is notable for the substantial extent of ongoing development and cyclic remodelling that occurs during pubertal and adult life, as the processes of germ cell maturation, secondary sex organ development and the capacity to support pregnancy are acquired. Transforming growth factor β1 (TGFβ1) has diverse roles in regulation of cell differentiation and proliferation in tissue development and repair. It is therefore unsurprising that descriptive studies and *in vitro* culture experiments have implicated TGFβ1

in almost every aspect of reproductive function. An opportunity to study the physiological significance of TGFβ1 *in vivo* is provided by the availability of TGFβ1 null mutant mice. TGFβ1 null mice suffer severe multifocal inflammatory lesions and do not survive past weaning age. However, rendering these mice immunocompromised prevents inflammation and permits TGFβ1 null mice to develop to healthy to adulthood. The aim of these studies is to examine both male and female reproductive function in TGFβ1 null mice on the immunocompromised *scid* background. We find that TGFβ1 is indeed a critical cytokine for reproductive success. Only 40% of TGFβ1 null females are able to mate with normal males, with 20% of these resulting in live litters born. TGFβ1 null males exhibited complete infertility. In both males and females, TGFβ1 is essential for functioning of the hypothalamo-pituitary-gonadal axis, with circulating luteinising hormone (LH) dramatically reduced, leading to downstream effects on testosterone production in males and cycling abnormalities in females. Ovarian function is further compromised, number of ovulations per cycle is reduced, and progesterone output per corpus luteum in early pregnancy is also diminished. Oocyte incompetence and early embryo arrest are observed. In addition to LH and testosterone deficiency, male TGFβ1 null mice demonstrate complete inability to mate with females, associated with failure to initiate and/or sustain successful penile intromission or ejaculation. These studies demonstrate the profound importance of TGFβ1 in male and female reproductive physiology.

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ENDOCRINE DISRUPTION AND THE DEVELOPMENT OF THE REPRODUCTIVE SYSTEM: LESSONS FROM WILDLIFE

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For over a century, wildlife and other animals have been used to predict the potential detrimental or beneficial human health effects of various environmental factors. These factors range from dietary and pharmaceutical effects to exposures to toxic chemicals. As the molecular, cellular and physiological mechanisms underlying the biology of reproduction in vertebrates are clarified, we are now able to determine which endpoints indicate compromised reproductive potential. Studies examining the reproductive biology of an wide array of vertebrate species, from fish to non-primate mammals, indicated that exposure to common environmental contaminants disrupts the development and functioning of the reproductive system. Specifically, developmental exposure to compounds such as various pesticides, plasticizers, industrial waste products, chemical stabilizers, pharmaceutics, plant secondary compounds (phytoestrogens) and flame retardants alter the development of the ovary or testis, for example, leading to compromises in reproductive potential. My talk will involve one example that involves the formation of an ovarian condition termed the multioocytic (multiovular) follicle in which an individual ovarian follicle has multiple oocytes, instead of a single oocyte that is normal. This condition has been associated with reduced fertility and an elevated frequency of death of early embryos. Several laboratories have now shown that this condition can be induced in laboratory rodents at high frequency following the exposure of the embryonic or neonatal female to chemicals with estrogenic action. Likewise, developing female alligators exposed to elevated concentrations of various pesticides exhibit a similar condition. Recent experimental studies have shown that this condition could be associated with altered functioning of a common pathway found in all vertebrate ovaries involving the activin-inhibin-follistatin signaling system. These data appear to correlate well with similar alterations reported in the DES daughter population. Although each species has its unique attributes, significant conservation exists in the underlying molecular, cellular and physiological systems associated with vertebrate reproduction, allowing us, with a reasonable degree of caution, to use data from wildlife and other animal species to access potential environmental influences on the process of human reproduction.

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A HIGH PHYTOESTROGEN DIET DISRUPTS MALE REPRODUCTIVE FUNCTION.

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Since phytoestrogens were first associated with disruption of mammalian fertility (Moersch et al. 1967), there has been considerable interest in their effects on development and function of reproductive tissues. Phytoestrogens are plant-derived compounds able to activate oestrogen receptors. They are common in human and animal diets, particularly from soy-based foods. Much effort has focused on effects of exposure to these agents during foetal and neonatal periods of development in the male. However, exposure to phytoestrogens in the adult male has received very little attention. Our studies have shown that acute exposure of adult male rats to a diet high in phytoestrogen content reduces the number of pups (litter size) they father compared with male rats who are fed a diet of low phytoestrogen content, possibly because of sperm damage due to increased oxidative stress (Glover and Assinder, 2006). Prolonged (chronic) exposure disrupts spermatogenesis with increased apoptosis of spermatocytes and round spermatids resulting in reduced sperm counts (Assinder et al., 2007). These effects are independent of the hypothalamus-pituitary-testicular axis and likely due to disruption of steroid regulation of the male reproductive tract.

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EXPRESSION AND ACTIVITY OF PHASE I DETOXIFYING ENZYMES IN THE MALE GERM LINE OF THE MOUSE.

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Members of the cytochrome P450 (P450) family are involved in phase I detoxification as well as normal cellular processes such as steroidogenesis. The testicular expression of steroidogenic P450s has been well documented. However a non-hepatic role for Phase I enzymes has yet to be fully elucidated. One possible outcome of the detoxifying process is DNA damage. We have examined the gene expression of, the P450 encoding, *cyp* genes in the male germ line. Utilising array technology we examined the expression of *cyp* genes in isolated germ cells as well as whole testis. Phase I P450s were expressed in all cell and tissue types examined. Real time PCR, in situ hybridisation, immunohistochemistry and Western blotting were all used to confirm the expression of several P450 genes. We also confirmed the recent finding that the steroidogenic *cyp17a1* is expressed in the germ line.

One phase I enzyme present in all germ cells was *cyp2e1*. Given the well-documented and unique role this enzyme has in the metabolism of acrylamide to glycidamide we assessed the ability of isolated germ cells to metabolise acrylamide. Previously acrylamide has been found to generate transgenerational toxicity in mice. These effects after acute dosing have generally been found to occur in late stage spermatogenesis. However the ability of early stage germ cells to metabolise acrylamide raises the possibility of chronic genotoxicity being possible. This has important consequences in our understanding of the cumulative effect of environmental exposure to xenobiotics.

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TRANSIENT ENDOCRINE DISRUPTION INDUCES PROSTATE PATHOLOGIES UPON AGING.

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Normal development and differentiation of the prostate gland is regulated by androgenic and estrogenic hormones requiring a complex interplay between endocrine and cell-cell signaling. Although prostate disease occurs in later life, it is known that transient perturbations in hormone action or in the relative ratio of androgens to estrogens, result in pathologies in late life - long after the initial event. We and others have demonstrated the importance of androgens and estrogens in the maintenance of the epithelial stem cell niche and the pivotal role of the stroma in mediating these effects.

The fungicide Vinclozolin is an anti-androgen and an endocrine disrupting chemical (EDC), with adverse effects on male reproductive tract development. We show transient neonatal exposure to Vinclozolin results in the perturbation of hormone action and aberrant stromal-epithelial cell signaling that alters the prostatic stem cell niche. Epithelial cell pathologies occur at maturity; specifically prostatic inflammatory atrophy. Since chronic inflammation is linked to the onset of premalignant lesions, these results provide a mechanism for the long range effects of transient exposure to Vinclozolin on the prostate gland.

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THYROID CANCER -ISSUES

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Thyroid cancer is being increasingly diagnosed in Eastern Australia (1) and is now the third most common cancer in females between 15 and 39 years of age. The natural history is long in most patients and management has always been largely based on retrospective data. There are many evolving issues in the diagnosis and management of thyroid cancer and this presentation will discuss some of them.

Diagnosis of Thyroid Cancer: The risk of malignancy in a thyroid nodule increases with age and serum TSH concentration. The latter is an independent predictor of malignancy (2) .

Non diagnostic FNAC: Nondiagnostic needle biopsies (less than 6 groups of 10-20 thyroid follicular cells per group on at least 2 slides) occur in about 15% of cases and are more likely if the nodule is small or cystic. Repeated biopsy under ultrasound guidance can increase the diagnostic rate up to 96.5% (3) .

Micronodules and microcarcinoma: Increasingly endocrinologists are asked to review patients in whom a small thyroid cancer has been noted incidentally during neck imaging for other purposes. Small, impalpable thyroid nodules become increasingly frequent with age (after age 40 incidence in percent is roughly age -10). If careful USS demonstrates features suggestive of malignancy (marked hypoechogenicity, intranodular vascularity, incomplete peripheral halo, irregular margin, central microcalcification or cervical lymphadenopathy) guided FNAC should be done (4) . Other cases can be observed.

The prevalence of papillary microcarcinoma is surprisingly high (up to 39%). This is very much higher than the clinically apparent prevalence, suggesting that the majority of these cancers are harmless.

Extent of surgery: There are several thyroid cancer prognosticating models that essentially divide patients into low and high risk groups. There is some surgical debate about less than total thyroidectomy for low risk cancers. Arguments for total thyroidectomy include better radioiodine ablation of residual thyroid tissue and better detection and treatment of locoregional and/or distant

metastases, measurement of serum thyroglobulin (Tg) becomes more sensitive, up to 85% of patients with papillary cancer have cancer in the contralateral lobe, recurrence in the contralateral lobe occurs in 7% of patients and total thyroidectomy reduces the admittedly low risk of dedifferentiation to anaplastic cancer.

Indications for postoperative radioiodine ablation: Powerful arguments for postoperative radioiodine ablation of all differentiated thyroid carcinomas of follicle cell origin other than papillary cancers less than 1.0-1.5 cm were put by Mazzaferri in 1994 (5) and again in 2001 (6) Accordingly most endocrinologists who manage thyroid cancer follow this advice. The 60 years Mayo Clinic experience suggests however that patients with good prognosis papillary cancer do not benefit from radioiodine ablation (7, 8) and a recent consensus statement also recommends a more conservative approach (8).

Follow up: The natural history of thyroid cancer is often long and recurrences can occur after many years. Follow up should therefore usually be life long (9) . Attention also needs to be paid to plasma calcium status and the effects of long term TSH suppression. Measurement of serum (Tg) levels has long been a key part of follow up. Tg in patients after total thyroidectomy and radioiodine ablation is a sensitive and specific marker of residual and recurrent cancer (10) . Tg secretion is increased by TSH and stimulated levels are more sensitive than those measured during thyroxine treatment. TSH stimulation can be done by thyroxine withdrawal or injection of recombinant TSH, although a careful meta-analysis suggests that accuracy is better after T4 withdrawal. A new highly sensitive chemiluminescent Tg assay may allow screening of many patients for recurrence without withdrawal of T4 or administration of rTSH (11) . A negative stimulated serum thyroglobulin and a negative neck USS indicates an excellent prognosis in papillary carcinoma, reducing the need for intensive follow up.

Up to 25% of patients with papillary carcinomas have autoantibodies to Tg that interfere with its measurement. There is not a good correlation between antibody titre and degree of interference and any antibody detected should induce suspicion about the accuracy of the assay. Most Australian laboratories use IMA methods to measure Tg and the presence of antibodies will always lead to falsely low Tg levels. Other methods for detecting residual disease (radioiodine scanning, USS, CT, PET etc) must be used in these patients.

Iodine is taken up into thyroid cells by the sodium iodide symporter (NIS). Loss of NIS expression by recurrent or metastatic disease is not uncommon and leads to failure of radioiodine uptake. The combination of measurable Tg and negative radioiodine scan indicates a need to proceed to alternative localising methods (thallium or MIBI scanning, USS, CT). PET scanning may be useful (12) .

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GENETICS OF AUTOIMMUNE THYROID DISEASE

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Susceptibility to the common autoimmune thyroid diseases, Graves' Disease and Autoimmune Hypothyroidism, appears to involve a complex interaction between genetic factors and the environment. Familial aggregation of the diseases provides evidence of heritability with analysis of twin data suggesting that up to 79% of the propensity to develop Graves' disease is attributable to genetic factors. The human leucocyte antigen (HLA) class II genes contribute to susceptibility with relative risks of 1.9-4.0 for Graves' disease and 1.9 for Autoimmune Hypothyroidism. Primary susceptibility has also been mapped to the CTLA-4 (cytotoxic T lymphocyte antigen 4) gene with relative risk of 1.5. More recently, one of the regulatory phosphatases, PTPN22 (protein tyrosine phosphatase, non-receptor type 22), has been established as having a role in autoimmune thyroid disease as well as susceptibility to other autoimmune disorders. Overall these genes account for only a small proportion of the heritability of autoimmune thyroid disease with other genes yet to be identified. These findings, together with results from genome screening in autoimmune thyroid disease, will be discussed.

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THYROID HORMONE REPLACEMENT

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The aims of thyroid hormone replacement therapy are to relieve symptoms of hypothyroidism and prevent associated complications. Despite the fact that thyroid replacement therapy has been used for over a hundred years, many fundamental issues remain unresolved, including diagnosis, indications for treatment, optimal treatment regimens, treatment targets and effect of treatment on associated disorders such as cardiovascular disease. Overt hypothyroidism is usually an indication for thyroid replacement therapy, but the benefits of treating subclinical hypothyroidism remain uncertain, largely because of an inadequate research base.

Thyroxine is standard treatment for hypothyroidism, but in some patients, symptoms of ill-health persist despite apparently adequate treatment. It is unclear whether this arises from comorbidity or because standard thyroxine replacement is in some way inadequate for some individuals. Combined thyroxine/T3 treatment is theoretically attractive as a replacement regimen, but randomised controlled trials have failed to show convincing benefits over thyroxine monotherapy. Some authorities recommend fine adjustment of thyroxine dosage until the serum TSH concentration is in the lower reference range (e.g. 0.4-2.0 mU/L), but in a randomised controlled trial we found no evidence that small changes in thyroxine dosage altered hypothyroid symptoms or quality of life.

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RECENT ADVANCES IN THYROID SURGERY

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Melbourne, and Monash University Endocrine Surgery UnitBenign thyroid nodules are common whilst thyroid cancer is rare and death from thyroid cancer even rarer. Preoperative clinical, ultrasonographic and cytological assessment determine which of the minority of thyroid nodules require thyroidectomy. Clinical suspicion of cancer, pressure symptoms retrosternal extension, thyrotoxicosis, family history of neck irradiation, recurrent thyroid cysts of greater than 4cm in diameter are indications for thyroidectomy.

We introduced synoptic cytology reporting in association with dedicated ultrasound guided fine needle aspiration cytology (FNAC) to facilitate directed management. We showed a significant reduction in the non-diagnostic rate of FNAC with ultrasound guidance ($P < 0.02$), which in our series is now 3.1% (world literature reports rates of up to 15%).

The five synoptic cytology reporting categories are non-diagnostic, benign non-neoplastic, indeterminate follicular lesions (of which up to 20% may be malignant), suspicious of malignancy, and malignant, the latter three mandating thyroidectomy.

In our series of 660 patients undergoing cytology, the accuracy of FNAC was 79.3%, sensitivity 79%, specificity 79%. The likelihood ratio of a malignant FNAC was infinity, there were no false positives and no false negatives for cancer in the benign non-neoplastic group (but a 20% false negative rate for benign follicular adenomas).

Traditionally subtotal thyroidectomy (STT) was the preferred option because of an incorrect perception of a lower complication rate of permanent recurrent laryngeal nerve palsy (0 – 1.3%)) and permanent hypoparathyroidism (1%). However, similar rates are widely reported following total thyroidectomy (TT). In my series of 336 total thyroidectomy patients, the permanent recurrent laryngeal nerve palsy rate was 0.3% and the permanent hypoparathyroidism rate was 1.8%. The disadvantages of STT for MNG are a high recurrence rate (between 30-50%), and increased risks of reoperation. STT for Graves' disease does not prevent hypothyroidism and does not avoid persistent disease. For thyroid cancer, TT enables treatment of multi-centricity of the primary tumour, enables radio-iodine scanning and therapy, enables follow up with serum thyroglobulin and suppressive therapy with thyroid hormones. Therefore TT removes the disease process completely, lowers recurrence rates for multi-nodular goitre, Graves' disease and thyroid cancer, avoids the substantial risks of reoperative surgery, at a similar complication rate to that seen following STT, and is now the preferred operation.

In our series, significant temporary hypocalcaemia developed in 13.4% of cases. Parathyroid auto-transplantation was performed in 12.8% and contributed to the low permanent hypoparathyroidism rate (1.8%).

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MEDIA FOR PRE-IMPLANTATION EMBRYO CULTURE ALTERS THE QUALITY OF RESULTANT ICM COLONIES IN THE MOUSE

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Mouse embryonic stem cells are routinely derived from in vivo developed blastocysts, while human ESCs are derived from excess ART embryos cultured in a variety of different culture conditions. Current efficiencies of generation of human ESC lines are low and cell lines show different growth and differentiation characteristics. It is known that culture of the early embryo can program fetal and offspring development, and we hypothesise that culture may similarly affect the isolation and the quality of ESCs. Thus the aim of this study was to compare different culture systems for pre- and post-compaction mouse embryo development, on embryo development and quality of subsequent inner cell mass (ICM) colonies.

Mouse zygotes were collected from superovulated F1 (C57Bl/6xCBA) female mice and cultured in a 2x2 factorial experimental design, comprising a simple media (sG1) or sequential culture media (G1/G2) for either pre-(48h) or post-compaction stage (48-96h) development. Embryo development and blastocyst quality were assessed at 48h and 96h of culture. Attachment and ICM outgrowth quality was assessed by culture of blastocysts in Glasglow MEM +5% FCS on gelatine coated plates for 96h.

Exposure of embryos to sG1 reduced embryo development at all stages (P<0.001). The percentage of ICM cells in the blastocysts was highest in sequential media G1/G2 (20.2±1.6). Exposure to sG1 at either the pre-compaction (16.4±1.6) and/or post-compaction (12.4±1.6) stage of development significantly reduced the percentage of ICM (P<0.05). Culture for the pre-compaction and/or post-compaction stage for 48h in sG1 lowered attachment and outgrowth rates compared to blastocysts derived in G1/G2 (P<0.01), and resulted in smaller ICM colonies with increased evidence of early stages of differentiation.

The culture environment for the early embryo affected not only the developmental ability of the embryo and number of ICM cells in the blastocyst, but also affected the efficiency and quality of the ICM colonies produced. This may provide some insight into the differential quality of human ESC's because the majority have been exposed to a simple media.

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PRODUCTION OF RABBIT BLASTOCYSTS BY NUCLEAR TRANSFER OF ADULT BONE MARROW MESENCHYMAL STEM CELLS

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Recent research indicates that mesenchymal stem cells (MSCs) isolated from porcine bone marrow are more efficient than fibroblasts for somatic cell nuclear transfer (SCNT) because of their less differentiation that led to easy reprogrammability to resemble the genome of the zygote (Faast et al. 2006 Cloning Stem Cells 8, 166-173). The aim of this study was to establish a practical protocol for somatic cell nuclear transfer by using MSCs isolated from rabbit bone marrow and to compare the developmental potential of cloned embryos with MSCs and cumulus cells (CCs) by assessing the fusion, cleavage and blastocyst rate. Matured oocytes from superovulated adult does were enucleated and inserted single MSCs or CCs (from passages 3-7) into the perivitelline space. After electrofusion by double DC pulses of 2.0 kV/cm for 20 m sec spaced 3 sec apart, the reconstructed embryos were activated by incubating for 4 min with 5.0 m M ionomycin in dark and culturing for 3 hours with 2.0 mM 6-DMAP in mM199 (TCM 199 supplemented with 10% FBS) and assessed for fusion at the end of 6-DMAP treatment (0 h). All fused complexes were cultured in vitro in mM199 under mineral oil in a humidified atmosphere of 5 % CO_2 in air at 38 °C . Half volume of fresh medium was replaced every 48 h. Cleavage (24 h) and blastocysts (120 h) development derived from the fused complexes were recorded. Fusion rate was significantly (P<0.05) lower in complexes reconstructed from MSCs than that from CCs (74/126; 58.7% vs. 33/45; 73.3%, respectively, 5 replicates). However, cleavage and blastocyst rates in fused complexes derived from MSCs (51/74; 68.9% and 14/74; 18.9%) did not differ from those derived from CCs (24/33; 72.7% and 9/33; 27.3%). In conclusion, the results suggest that bone marrow MSCs can be an attractive cell type as donor cells and have a similar potential for producing cloned rabbit blastocysts when comparing with cumulus cells.

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FUNCTIONAL CHARACTERISATION OF THE CUMULUS OOCYTE MATRIX DURING MATURATION OF OOCYTES

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Oocyte maturation initiated by the LH-surge requires a cascade of gene expression within the ovarian follicle including production of a unique extracellular matrix by the cumulus cells surrounding the oocyte. The *in vitro* maturation (IVM) of cumulus enclosed oocytes offers health benefits for patients undergoing assisted reproduction, however, IVM oocytes have low competence and are sensitive to high glucose in the maturation medium. We found that two matrix factors; versican and the protease Adamts1 had >10-fold reduced mRNA and protein abundance (P<0.05 ANOVA) in mouse cumulus oocyte complexes (COCs) after IVM in αMEM containing 15.5mM glucose and stimulated with Egf and/or FSH. Significant induction of *Has2* expression (8-fold, P<0.05) over nonstimulated controls confirms the COC response to IVM conditions. We also identified *VCAN* and *ADAMTS1* expression in human *in vivo* matured cumulus cells demonstrating the relevance of these matrix proteins in human fertility.

Although cumulus matrix expansion is widely considered a key indicator of oocyte developmental potential, the function of the COC matrix in oocyte maturation is unknown. We assessed properties of the COC matrix by examining transport of fluorescently labelled glucose and cholesterol using confocal imaging and quantitative measurement of metabolite transport across the diameter of each COC. This demonstrated profound differences in the control of metabolite supply to oocytes in IVM vs *in vivo* matured complexes. Cumulus cells and oocytes of IVM COCs readily took up glucose and cholesterol, while *in vivo* matured COCs excluded glucose from the entire COC and cholesterol was excluded from oocytes resulting in inverse gradient patterns of glucose and cholesterol abundance for IVM vs *in vivo* maturation conditions. This study is the first to show that commonly used IVM conditions result in altered gene expression in the cumulus cells. Of considerable importance we show that the COC matrix has a molecular filtration properties that may control metabolite supply to the oocyte potentially explaining the poor competence and sensitivity to glucose of IVM oocytes.

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EPIGENETIC CHANGES IN THE MURINE OOCYTE AND EMBRYO FOLLOWING MATERNAL DIETARY PROTEIN SUPPLEMENTATION

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With our growing awareness of lifestyle factors such as diet, and how these factors interact with reproductive processes, comes the understanding of potential programming of the health and development of subsequent offspring. An emerging hypothesis involves permanent epigenetic changes that take place during early development, and which are postulated to be mechanisms responsible for programming such life-long effects. Imprinted genes are regulated by the process of DNA methylation and are established during oocyte growth, via DNA methyltransferase (Dnmt) enzymes. In this study the consequence of maternal dietary protein supply prior to conception during oocyte development was examined, and subsequent epigenetic indicators investigated in the oocyte and early embryo. 5 week-old CBA/C57Bl6 hybrid female mice were fed high (25%=HPD), medium (14%=MPD) or low (9%=LPD) levels of dietary protein for 3-4 weeks, prior to gonadotrophin administration and oocyte or embryo recovery. Global methylation was examined in the 2-cell embryo (40hrs post-hCG) using a primary monoclonal antibody to 5-methyl-cytidine, detected using a FITC-conjugated secondary antibody and analysed using confocal microscopy. Gene expression of DNA methyltransferase (Dnmt) enzymes and the imprinted IGF2R gene was examined in pools (n=20) of germinal vesicle oocytes and in 2-cell embryos, using real-time PCR. Antibody staining revealed greater global methylation in 2-cell embryos recovered from LPD mothers relative to MPD females (1.62 vs 1.00;$p<0.05$), and this trend persisted for the HPD group (1.61; $p>0.097$). Concomitant with this change in methylation in the embryo, IGF2R expression tended to be lower in GV oocytes from females fed the LPD (0.23;$p=0.05$) or HPD (0.43;$p=0.096$) relative to the MPD control oocytes (1.0). Furthermore, expression of Dnmt3l and 3b, known to be involved in methylation imprint establishment also tended to be less in GV oocytes from LPD females ($p<0.08$). These results support the notion that oocyte growth is an important developmental period for determining expression of the imprinted gene IGF2R and that this period may be susceptible to alterations in maternal dietary protein supply.

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PRODUCTION OF MONOZYGOTIC TWIN LAMBS FROM SEX-SORTED SPERM BY EMBRYO BISECTION

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Insemination with X- or Y-chromosome bearing sperm separated by modified flow cytometry remains the most effective method for producing offspring of a pre-determined sex. The efficiency of producing embryos from sex-sorted sperm in a multiple ovulation and embryo transfer (MOET) programme may be further increased by incorporating embryo bisection. The aim of this study was to determine the effect of bisection on the *in vitro* and *in vivo* survival of embryos produced from sex-sorted and non-sorted sperm. Sperm frozen after flow cytometric sorting (X- and Y-bearing) or semen collection (non-sorted) was thawed immediately prior to AI. Superovulated donor ewes were inseminated laparoscopically with either 15 million non-sorted sperm, 5 or 15 million X-bearing sperm, or 5 or 15 million Y-bearing sperm. On Day 6 after AI, embryos were recovered via mid-ventral laparotomy and bisected using a hand-held embryo splitting blade. Each pair of demi-embryos was either transferred to a synchronized recipient ewe or cultured *in vitro*. There was no effect of sperm sex or dose on fertilization rate or embryo development. From 29 embryos bisected and cultured *in vitro*, 44 demi-embryos reformed expanded blastocysts, representing a 52% increase in the number of embryos originally recovered. Staining at 48h showed that the demi-embryos had a mean of 77.3 ± 7.4 cells. *In vitro*, a greater proportion of embryos produced from the 15 million dose survived bisection compared with the 5 million dose (86% v 60%; $P<0.05$). Ultrasonographic pregnancy diagnosis at 55-60d gestation revealed an *in vivo* survival rate of 68%. From 28 pairs of demi-embryos transferred to 28 recipient ewes, 15 pregnancies were established: 11 singletons and 4 sets of twins. All but one singleton pregnancy was carried to term. To our knowledge, these are the first monozygotic twin lambs produced from sex-sorted sperm by embryo bisection. Embryo bisection may be a useful way of increasing the number of embryos for transfer if a modest increase in the rate of demi-embryo survival can be achieved.

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DISRUPTION OF BI-DIRECTIONAL OOCYTE-CUMULUS PARACRINE SIGNALLING DURING IN VITRO MATURATION REDUCES SUBSEQUENT MOUSE OOCYTE DEVELOPMENTAL COMPETENCE

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Oocyte-cumulus cell (CC) bi-directional communication is essential for normal development of the oocyte and cumulus cells within the follicle. CCs relay FSH and EGF endocrine signals to the oocyte while oocyte paracrine factors such as growth differentiation factor 9 principally mediate their effects on CCs through the SMAD2/3 pathway. This study aimed to observe the effects of disrupting either signalling pathway during oocyte *in vitro* maturation (IVM) on oocyte health and developmental competence.

Cumulus-oocyte complexes (COCs) were aspirated from antral follicles of pre-pubertal (CBAB6F1) mice at 47h post eCG and matured at 37 ° C in 6%CO_2 5%O_2 for 17h in Waymouth's medium+5% serum, with or without 50mIU/ml FSH and 10ng/ml EGF, 4

m M of the SMAD2/3 inhibitor SB-431542, or its 0.04% DMSO control. COCs were then analysed for levels of oxidative stress using redox sensor red (~50/treatment) or fertilised and cultured (150-200/treatment) to the blastocyst stage in G1.2/G2.2 media and blastocyst cell numbers were assessed.

SMAD2/3 inhibition or absence of FSH/EGF during IVM significantly decreased fertilisation rates compared to controls (62% vs 80%; 69% vs 78%, respectively; $P<0.05$). No differences were observed in the percentage of blastocysts or hatching blastocysts from cleaved embryos with SB-431542. However, blastocyst development was significantly reduced in the absence of FSH/EGF compared to maturation with FSH/EGF (50% vs 73% respectively; $P<0.05$). IVM with SB-431542 or without FSH/EGF both significantly ($P<0.001$) decreased blastocyst inner cell mass percentage (28% vs 37% control; 18% vs 32% control respectively; $P<0.001$). Oxidative stress levels were substantially higher ($P<0.001$) in CCs and denuded oocytes matured with SB-431542 (6 and 4 fold increase, respectively) or in the absence of FSH/EGF (5 and 4 fold increase, respectively) relative to controls.

These results demonstrate that disruption of either, oocyte paracrine communication to CCs or CC to oocyte signalling during IVM, perturbs oocyte and CC health which impacts blastocyst quality. Therefore, maintenance of this communication loop is a critical component of oocyte and embryo developmental competence.

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THE CO-OPERATIVE EFFECT OF GDF9 AND BMP15 ON GRANULOSA CELL FUNCTION IS MODULATED PRIMARILY THROUGH BMP RECEPTOR II

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Growth and differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP15, GDF9B) are oocyte–derived proteins essential for the growth and function of ovarian follicles. Moreover, ovine (o) GDF9 and oBMP15 co-operate to increase both ^{3}H-thymidine incorporation and α-inhibin production, and to inhibit progesterone production by rat or ovine granulosa cells. Although the receptors through which these proteins act individually have been determined, the receptor(s) involved in mediating the co-operative effects of GDF9 and BMP15 is (are) unknown. In this study, the effects of the extracellular domains of the Type I and Type II TGFβ receptors on stimulation of rat granulosa cells ^{3}H-thymidine incorporation by oGDF9 and oBMP15 were investigated. Stimulation of ^{3}H-thymidine incorporation was completely blocked by the BMP receptor II (BMPRII) extracellular domain but unaffected by any other Type II and all of the Type I receptors. These results suggest that the initial interaction of oGDF9 and oBMP15 is with BMPRII and that a type I receptor is either recruited or already associated with BMPRII in order to mediate the cooperative effects of GDF9 and BMP15.

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MITOCHONDRIAL CONSEQUENCES FOLLOWING *IN VITRO* MATURATION (IVM) OF MOUSE OOCYTES IN VARYING OXYGEN CONCENTRATIONS.

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The effects of oxygen concentration during IVM on oocyte metabolism are unknown, despite the oocyte's dependency on oxidative phosphorylation for ATP production. The aim here was to investigate effects of O_2 availability during maturation on the function and distribution of oocyte mitochondria. I mmature cumulus oocyte complexes (COCs) were collected from the ovaries of eCG-stimulated CBAB6F1 females (21 d) and matured for 17-18 h under 2, 5, 10 or 20% O_2. To detect regional differences in mitochondrial membrane potential, oocytes were stained with 1.5 mM JC-1 (5,5'6,6'-tetrachloro-1,1,3,3'-tetraethylbenzimidazolycarbocyanine iodide). Confocal images were used to calculate the mean pixel intensity of red (high membrane potential) and green (low membrane potential) fluorescence in 3 different areas of the oocyte - perinuclear, intermediate and cortical regions. Oocytes matured at 5% O_2 had a significantly lower red to green pixel ratio in all three areas, compared to 2, 10 and 20% O_2 treated oocytes. The 2, 10 and 20%, but not 5% O_2, treated oocytes exhibited a significant ($P<0.05$) increase in red to green pixel ratio in the cortical region, compared to the other two regions. Spatial distribution of all mitochondria was also assessed using Mitotracker Green with analysis of green fluorescence in the 3 regions, in oocytes matured at 2, 5 or 20% oxygen and in in vivo matured oocytes, collected post hCG. For Mitotracker Green analysis, the cortical to perinuclear ratio was increased ($P<0.05$) in all IVM oocytes compared to in vivo matured oocytes. Furthermore, this ratio was increased within oocytes matured in 2% ($p<0.05$) and 20% ($p=0.06$) compared with 5% O_2. This data suggests that more mitochondria were distributed to the outer edge of the oocyte following IVM, but that 5% O_2 perturbed this distribution. These results demonstrate that IVM oocytes have an altered pattern of mitochondrial distribution and/or function and this was affected by oxygen concentration during IVM. This is possible evidence of altered metabolism in IVM, which is O_2 dependent.

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A QUANTITATIVE ULTRASTRUCTURAL STUDY OF EARLY STAGE OOCYTES FROM WILD-TYPE AND BOOROOLA SHEEP

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In the sheep ovary, primordial follicles are formed as an oocyte surrounded by either a single layer of flattened granulosa cells (Type 1) or a mixture of flattened and cuboidal granulosa cells (Type 1a). Booroola (BB) sheep have a mutation in the growth factor receptor, activin-like kinase receptor 6 (ALK6) which is expressed in oocytes from the Type 1 stage of growth. In homozygous BB ewes, oocytes undergo precocious maturation that appears to be initiated during the preantral growth phase. The aim of this study was to quantify the ultrastructural features of oocytes from BB and wild-type (++) ewes in Type1/1a follicles. Ovaries from 6 ++ and 5 BB 4 week old lambs were processed for both light microscopy (LM) and electron microscopy (EM). LM and stereological methods were used to estimate the mean volume of the oocytes from each genotype. EM images and point counting or linear intercept counting were used to estimate the volume of smooth endoplasmic reticulum, mitochondria, Golgi, vesicles, lipid, ribosomes and zona pellucida and the surface area of the outer and inner mictochondrial membranes, microvilli and cell junctions. The Type 1/1a BB oocytes had a greater volume than the ++ oocytes (BB: 29.76 +/-0.58 vs ++: 27.05 +/- 0.30; $P<0.01$). In Type 1/1a BB oocytes, the smooth endoplasmic reticulum, mitochondria and zona pellucida volumes were greater than in ++ oocytes ($P<0.01$) as were the surface areas of the outer and inner mitochondrial membranes, oocyte membrane, and zonula adherens type junctions ($P<0.05$). These results suggest that the ALK6 mutation advances the development of oocytes in BB primordial follicles.

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INSULIN RESISTANCE TESTING – CLINICAL AND COMMUNITY UTILITY

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The 1959 discovery of the technique of radio-immunoassay by Yalow and Berson allowed the measurement of insulin in blood for the first time, leading to an explosion in knowledge of diabetes and other endocrine diseases, confirming that type 1 diabetes and type 2 diabetes (T2D) are separate diseases. However, the only clinical use of the insulin assay was in the diagnosis of pancreatic insulin-secreting tumours.

Over the past 30 years, studies have shown that insulin resistance with compensatory hyperinsulinaemia is important in the causation of vascular disease, hypertension and a majority but not all cases of the polycystic ovary syndrome (PCOS), and also precedes the development of type 2 diabetes by decades.

The accurate measurement of insulin resistance is a complex, invasive and labour intensive research tool that measures insulin resistance in a stimulated state and which is not suited to clinical practice. So-called "surrogate" or clinical markers of insulin resistance have previously not had the same precision as the research techniques but can measure insulin resistance in the baseline state, reflecting hepatic insulin resistance, and in the stimulated state, reflecting muscle insulin resistance. Mathematical computations involving fasting insulin and glucose, such as the HOMA index, have now been shown to have a high correlation with the research measures. Sex hormone binding globulin levels in the reproductive decades also correlate well with the research techniques, as does the 0-3 hour area under the insulin curve on glucose tolerance testing in PCOS.

Insulin resistance testing will identify individuals at increased risk of T2D long before the onset of impaired glucose tolerance or impaired fasting glucose, allowing earlier intervention. Insulin resistance testing can also assist in the differentiation of the causes of PCOS. Insulin levels can be an effective motivational tool in lifestyle improvement in people at increased risk of development of T2D.

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MEASURING SERUM INSULIN AS A MARKER OF INSULIN RESISTANCE DOES NOT HAVE CLINICAL AND DIAGNOSTIC UTILITY.

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It is becoming increasingly clear that there is a continuum of lifestyle related diseases from physical inactivity and/or obesity through the progression of insulin resistance to include PCOS and gestational diabetes (in women), pre-diabetes, diabetes and CVD. As the epidemic of obesity continues, the clinical implications of related insulin resistance continue to expand. Yet there are currently no guidelines/ recommendations to support the measurement of insulin resistance in the clinical setting and this is for good reason.

The rationale for not measuring IR in the clinical setting is presented and addresses issues including:

The accuracy of clinically available measures of insulin resistance

The difficulties in defining insulin resistance

The clinical utility of insulin measurements
- prediction of clinical outcomes
- prediction of therapeutic responses
The alternatives to measuring insulin resistance
The future

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BIOMEDICAL AND AGRICULTURAL APPLICATIONS OF PORCINE CLONING

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The development of somatic cell nuclear transfer (SCNT) or cloning as it is more commonly known has allowed a number of biomedical as well as agricultural applications to be explored in the pig. SCNT can be used to produce genetically modified pigs for xenotransplantation research and has the potential to greatly improve productivity in the pig industry. Research into xenotransplantation requires the pig to be genetically modified to overcome immunological rejection as well as other barriers . The first step in the rejection pathway, hyperacute rejection, can be overcome by producing pigs in which the gene for α1,3 galactosyltransferase (Gal) has been knocked out. Gal produces an epitope to which human antibodies bind to initiate rejection. We and other groups have produced heterozygous Gal knockout pigs using gene targeting of somatic cells and SCNT. We have also used SCNT to produce CD39 transgenic pigs as part of a strategy to overcome coagulopathy seen following transplantation. By transfecting fibroblasts and then screening these for expression prior to SCNT it is possible to guarantee that all animals produced using this method express the transgene as well as transmit it to the next generation. SCNT also provides a method whereby elite animals contained within nucleus herds can be used at the commercial level. The major drawback to the commercial use of this technology at present is the relatively low efficiencies with which pigs can be cloned. Cloning is a multi-step process and improvements in all steps will be needed to improve overall efficiencies before this technology can be used commercially. Current research in our laboratory is focussed on identifying novel cell types including stem cells, which can increase these efficiencies as well as developing the necessary technologies such as embryo freezing to facilitate commercialisation.

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NUCLEAR TRANSFER IN CATTLE: A REPRODUCTIVE TOOL TO GENERATE ANIMALS FROM AN EXISTING OR MODIFIED GENOME.

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Nuclear transfer (NT) cloning creates an animal that is a genetic copy of the donor cell genome used in the procedure. Cloning technology provides new opportunities for livestock agriculture and biomedicine by either replicating existing embryonic or adult genotypes, or generating animals with novel genotypes by using genetically modified cells for NT. Improvements in the production systems and efficiency of cattle cloning include the embryo reconstruction methodology, the choice of donor cell line and cell cycle stage, recipient cytoplasmic environment, mode of activation and embryo aggregation. Nonetheless, the process remains inefficient. Presently, in cattle, about 10% of NT embryos result in long-term surviving clones. The low rates are probably due to incorrect epigenetic reprogramming of the donor genome following NT, leading to inappropriate patterns of gene expression during development which contribute to the high mortality and aberrant phenotypes observed throughout gestation, parturition and post-natally. Importantly, it appears that these clone-associated phenotypes are not transmitted to progeny following sexual reproduction with clones. This indicates that they represent epigenetic errors which are corrected during gametogenesis. Whilst this requires molecular confirmation, it provides confidence for the first practical application of NT in agriculture; namely, the production of cloned sires from genetically elite males for natural mating, to effectively disseminate genetic gain. More efficient NT methods will be required for larger scale dissemination of whole genotypes of elite livestock from nucleus herds directly to commercial producers. NT could increase the accuracy of animal selection in breeding schemes by evaluating the phenotype of different lines of cloned animals, obviating the need for progeny testing. When combined with embryonic cloning this would also reduce the generation interval, increasing the rate of genetic gain. Advances in animal genomics aid the ability to genetically modify cultured cells prior to NT, enabling the generation of livestock with enhanced production attributes or which produce superior quality food and biomedical products for niche markets. A significant challenge for the integration of this technology into farming systems includes regulatory and consumer acceptance, demanding effective science communication.

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NUCLEAR-MITOCHONDRIAL INTERACTIONS IN CLONED ANIMALS

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Mitochondria have a broad range of functions in cellular metabolism, cell signalling and programmed cell death. Mitochondrial biogenesis is under dual genetic control and requires extensive interaction between biparentally inherited nuclear genes and maternally inherited mitochondrial genes. Standard somatic cell nuclear transfer (NT) procedures deprive an oocytes' mitochondrial DNA (mtDNA) of the corresponding maternal nuclear DNA and force it to interact with an entirely foreign nucleus that is already interacting with foreign somatic mitochondria. As a result, most NT embryos, –foetuses, and –offspring carry somatic cell mtDNA in addition to recipient oocyte mtDNA. The consequences of this artificial heteroplasmy and, potentially, mtDNA recombination are at present unknown. Nuclear and mitochondrial genes have co-evolved and transmitochondrial cell culture and animal models predict functional incompatibilities in xenomitochondrial NT systems that increase with phylogenetic distance. It is therefore not surprising that interspecies NT approaches have thus far only been successful with species that also yield hybrid offspring after natural mating. We have detected significant tissue-specific reductions in mtDNA in intra-species NT foetuses as compared with *in vitro* and *in vivo* fertilized controls. This mtDNA depletion could reflect perturbed nuclear-mitochondrial interactions and provides a novel perspective on clone failure. MtDNA haplotype effects of recipient oocytes on intra-species NT efficiency have been correlated with differences in cytochrome c oxidase transcript abundance and ATP content of oocytes, and the cellular metabolism of NT foetuses. We have recently confirmed significant mtDNA effects on embryo-foetal development and placentation in a non-cloned transmitochondrial animal model. In conclusion, mtDNA effects and nuclear-mitochondrial interactions are likely to affect NT efficiency and outcomes. Functional constraints in nuclear-mitochondrial interactions have to be overcome for application of interspecies NT technology in conservation of endangered species or cell therapy.

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BOVINE EMBRYONIC STEM CELLS: ISOLATION, CHARACTERIZATION AND POTENTIAL APPLICATIONS

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The derivation of stem cells from domestic species is of interest due to their potential uses in animal reproduction and agricultural biotechnology. We have isolated bovine embryonic stem cells (bESCs) from *in vitro* produced blastocysts. The cells have been maintained in culture for 24 passages (>220 days of culture). They retained phenotypic and morphological characteristics of pluripotent cells with a small cytoplasmic/nuclear ratio and containing lipid inclusions in cytoplasm and nuclei with multiple nucleoli. Under appropriate conditions *in vitro* the cells form embryoid bodies, which expressed genes of pluripotency including Oct-4, Rex-1, Sox-2 and SSEA-1 and the three germ layers; Ectoderm (Pax-6, Vimentin, b-3 tubulin, Nestin, FGF5 & Nodal), Mesoderm (Lipoprotein lipase, Connexin 40, BMP4, Flk-1 & z-globin) and Endoderm (Gata-4, Gata-6, Somastatin, a-feto-protein, Transthyretin & Albumin). Significantly, when transplanted into SCID mice the bESCs had the ability to form teratomas *in vivo*. This is the first report on derivation of ESCs with *in vitro* and *in vivo* differentiation potential, in a commercially valuable species. We also report the isolation of putative stem cells from adult bovine skin biopsies, which express the stem cell markers Oct-4 and SSEA-1 analyzed by RT-PCR. Further, the cells are capable of forming spheroid structures of exclusively mesodermal origin and exhibit clonogenicity 70-77%. In summary, we have identified populations of stem cells from both embryonic and adult bovine tissues. Preliminary characterization of the cells suggests the cells display varying differentiation potential and the potential biotechnology applications of each will be discussed.

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IMMUNOSUPPRESSIVE PROPERTIES OF A SYNTHETIC PEPTIDE ANALOGOUS TO HUMAN SYNCYTIN IMMUNOSUPPRESSIVE PEPTIDE

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Background: Syncytin, a human endogenous retrovirus envelope protein encoded by the human endogenous retrovirus (HERV-W), is expressed in the placenta and has fusogenic properties. It is thought that Syncytin plays a critical role in human placental morphogenesis by meditating cytotrophoblast fusion to form the syncytiotrophoblast. It has been hypothesized that a putative immunosuppressive peptide sequence (ISU) within the transmembrane (TM) subunit of the protein may also play a role in suppressing the maternal immune response against the fetus. We therefore decided to test the immunosuppressive properties of a synthetic peptide analogous to the Syncytin ISU sequence.

Methods: Antibodies were raised to synthetic Syncytin ISU peptide. Human peripheral blood mononuclear cells were cultured for 24 hours at 37^{o}C (5% CO2) with maximal stimulating doses of lipopolysaccharide in the presence and absence of different concentrations of the Syncytin ISU peptide with and without different dilutions of the antibodies raised against the peptide. Following culture, the supernatant was removed and TNF-alpha levels were determined using ELISA.

Results: Our preliminary results showed a decrease in the production of TNF-alpha in the presence of the Syncytin ISU peptide. This effect was blocked by the addition of antibodies against this peptide

Conclusions: The putative immunosuppressive peptide of human Syncytin has immunosuppressive properties which could be relevant for immunoregulation during pregnancy.

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PRELIMINARY DEFINITION OF THE MECHANISMS BY WHICH ACTIVIN A IS RELEASED FROM CELLS AND ITS RESPONSE TO AN INFLAMMATORY STIMULUS

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Activin A is a pleiotrophic protein synthesised in many cell types, with reproductive, neurological and embryogenic functions, amongst others. We have shown that activin concentrations increase rapidly in the circulation following stimulus with inflammatory agents such as lipopolysaccharide (LPS; 1). Cells such as monocytes increase synthesis of the activin gene following LPS, resulting in increased production of activin A (2). Nevertheless, there is a paucity of information on the mechanism through which activin A is synthesised and released by cells under basal conditions or in response to inflammatory stimuli such as LPS. Therefore, we used blockers of protein secretory pathways to examine how activin A is released from cells. We first confirmed by modelling that activin uses a regulated secretory pathway employing a signal peptide motif as opposed to constitutive release. Blockade of endoplasmic reticulum (ER)/Golgi transport using brefeldin or monensin in endothelial, macrophage or Sertoli cell lines prevented release of activin A. However, the activin was retained in cells confirming that synthesis was unaffected but packaging and trafficking to the membrane was blocked. We chose to focus on the TM4 Sertoli cell line, as these cells release activin constitutively and respond rapidly (within 5 hours) to LPS treatment. Many proteins responsive to inflammation utilise the nuclear factor-κB (NF-κB) pathway. Inhibitors of NF-κB suppressed both constitutive and LPS-stimulated release of activin. Lipid raft inhibitors also modulated activin release with or without LPS, suggesting that activin is released via glycolipid-enriched membrane complexes. Lastly, an inhibitor of protein kinase C suppressed both constitutive and LPS-stimulated release of activin. Overall, these studies show that activin A is part of a sorting process involving the ER/Golgi and lipid rafts and that its secretion is regulated via protein kinase C and NF-κB in TM4 cells. Further delineation of these secretory and regulatory pathways will provide new information on how activin A reacts to inflammatory and other stimuli and is released rapidly from responsive cell types.

(1) Jones et al.(2004) J. Endocrinol 182, 69-80
(2) Erämaa et al.(1992) J. Exp. Med. 176, 1449-1452.

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EXPRESSION OF THE MURINE BETAGLYCAN GENE IN THE FOETAL LEYDIG CELLS DURING GONADOGENESIS IN THE MOUSE.

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Betaglycan has been identified as a co-receptor that binds both transforming growth factor-beta (TGF β) and inhibin with high affinity and is an important modulator in the activities of multiple TGF β superfamily members. Betaglycan is abundantly expressed in the adult testis and ovary, suggesting key roles in the regulation of reproduction in both sexes, but little is known about this receptor during gonadogenesis. In this study, we characterised the gonadal expression pattern of the betaglycan gene during murine development. Real-time RT-PCR analysis on testis and ovary samples revealed that betaglycan mRNA was differentially expressed, displaying higher expression in the developing testis between 14.5-16.5 days post-coitum (dpc). In situ hybridisation showed that betaglycan mRNA was predominantly expressed within the foetal testis interstitium but was strongly upregulated within the seminiferous cords after birth. Immunohistochemistry also confirmed that betaglycan protein was predominantly localised to the

interstitial cells surrounding the developing seminiferous cords. Double immunofluorescence assays showed betaglycan protein to be largely localised in cells also expressing Cyp11a (p450 Scc) within the foetal testis interstitium, indicating that betaglycan is expressed in foetal Leydig cells. In contrast, betaglycan expression was low in the foetal ovary but was highly expressed in the oocytes and granulosa cells from the day of birth onwards. The differential expression pattern of betaglycan in the male and female gonads during murine development, suggests multiple roles for betaglycan in gonadal differentiation and maturation. Supported by the NHMRC of Australia (RegKeys 338516 and 198705) and the Australian Research Council.

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INCREASED LUTEINISING HORMONE RESPONSE TO KISSPEPTIN DURING THE ANESTROUS SEASON IN EWES

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Kisspeptins are endogenous products of the Kiss1 gene that bind to the receptor, GPR54. Kisspeptins play a vital role in the regulation of gonadotrophin-releasing hormone (GnRH) and gonadotrophin secretion. Centrally or peripherally administered kisspeptins stimulate gonadotrophin secretion, in a GnRH dependant manner. In sheep, reproductive activity is seasonal, being activated by short-day photoperiod and inhibited by long days. During the non-breeding (anestrous) period, expression of Kiss1 mRNA in the hypothalamus is reduced[1]; moreover, kisspeptin treatment can induce ovulation during anestrous[2]. We examined whether the response to kisspeptin treatment is also varied between the breeding and non-breeding seasons. Corriedale ewes were treated with a submaximal intravenous dose (20 μg) of murine kisspeptin (or vehicle) during either the anestrous period (n=5) or during the luteal phase of the estrous cycle in the breeding season (n=6). Blood samples were collected every 10 min for 1 h prior and 2 h following treatment. LH concentration was determined by radioimmunoassay. We found that kisspeptin treatment increased the concentration of LH in all animals compared to vehicle treated controls. The response to kisspeptin was significantly (P<001) greater in ewes during the anestrous period. Area under the curve analysis revealed that the kisspeptin response was approximately 4-fold greater in the anestrous period (154±27) compared to luteal phase ewes (40±11). These data indicate, for the first time, differences in the gonadotropic response to kisspeptin in different functional states of the female reproductive axis of the ewe. The unexpected result that response is heightened in the anestrous season warrants further investigation. Studies are planned to assess the hypothalamic expression of GPR54 as well as the response to kisspeptin over the entire estrous cycle in the ewe. Supported by NHMRC Australia.

(1) Smith et al. (2007) Endocrinology 148:1150-1157.
(2) Caraty et al. (in press) Endocrinology.

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PREGNANCY AND NUTRITION DIFFERENTIALLY REGULATE EXPRESSION OF IGF-I AND IGF-II IN THE GUINEA PIG

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Maternal plasma IGF-I and IGF-II are elevated throughout pregnancy in humans and guinea pigs. Both IGFs are thought to alter partitioning of nutrients between the mother and conceptus, while IGF-II promotes trophoblast invasion and differentiation. To identify the effect of pregnancy and nutrition on maternal plasma IGF-I and —II and their potential sources, we measured their mRNA abundance in maternal tissues of ad libitum (AL) and feed restricted (R) (70% AL) guinea pigs in late gestation.

Pregnancy increased IGF-I mRNA in liver (p<0.001, ~ 4 fold), gastrocnemius (p=0.019, ~ 3 fold), parametrial fat (p<0.0001, ~ 11 fold), and IGF-II mRNA in the kidney (p= 0.05, ~ 6 fold) in AL and that of IGF-I in gastrocnemius and bicept similarly in R (p<0.05). Pregnancy decreased IGF-I mRNA (p<0.001, ~ 8 fold) and increased IGF-II mRNA (p<0.0001, ~9 fold) in the uterus and that of IGF-II in retroperitoneal fat (p=0.02, ~ 2 fold). Plasma IGF-I correlated positively with hepatic IGF-I mRNA (liver) but quadratically (r^2= 0.68, p<0.002), such that plasma IGF-I plateauing at higher levels of IGF-I mRNA. Plasma IGF-I also correlated positively with IGF-I mRNA in parametrial fat (r^2= 0.24, p = 0.008), gastrocnemius (r^2 = 0.12, p= 0.036) and biceps (r^2=0.65, p=0.001). The increased IGF-I expression in maternal liver, parametrial fat, gastrocnemius and biceps in late pregnancy and close relationship with plasma IGF-I in the fed and undernourished states implicates these tissues as potential sources of increased circulating IGF-I in the mother. The pregnancy related switch from IGF-I to IGF-II expression in the uterus suggests a potential role in regulation of placental function in late pregnancy. Factors regulating tissue IGF-I mRNA expression in late pregnancy are unknown, but the co-ordinate changes in pregnancy and undernutrition suggest they may be partly systemic in nature.

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POTENTIAL ROLE OF CRH IN MEDIATING IMMUNE FUNCTION IN PREGNANCY

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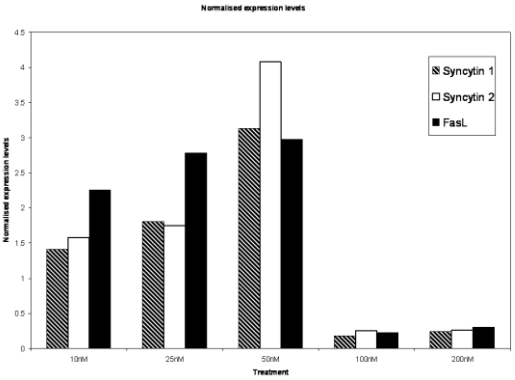
Background: Corticotrophin Releasing Hormone (CRH) may have important immunoregulatory roles during pregnancy stimulating the production of Fas Ligand (FasL). Syncytin, a human endogenous retrovirus envelope protein encoded by the human endogenous retrovirus (HERV-W), is expressed in the placenta and has fusogenic properties. Syncytin has been shown to play a critical role in human placental morphogenesis by mediating cytotrophoblast fusion to form the syncytiotrophoblast as well as a hypothesised immunosuppressive role during pregnancy. Syncytin can be upregulated at the mRNA and protein level by Forskolin, a cAMP inducer. CRH is an inducer of cAMP and is expressed at a number of sites including the placenta. We hypothesised that CRH stimulates the induction of Syncytin mRNA and also the immune mediator FasL.

Methods: BeWo cells were cultured at 37°C in HAM-F12 medium supplemented with 5% foetal calf serum in a humidified atmosphere of 5% CO^2 and 95% air. Cells were subcultured and grown for 1 day to 50% confluence. At 50% confluency, the medium was changed to one containing CRH (10, 25, 50, 100 or 200nM) or vehicle (saline) and incubated for 24 hours. RNA was then extracted from these cells and cDNA generated prior to quantitative RT-PCR analysis using 18s rRNA as an internal control.



Results: Real-time PCR analysis of treated BeWo cell cDNA showed an upregulation in Syncytin 1, Syncytin 2 and FasL mRNA following CRH treatment in a dose dependent fashion until 100nM. Higher concentrations appeared to inhibit this increase.

Conclusions: Using RT-PCR we showed that CRH is able to upregulate the expression of Syncytin 1, Syncytin 2 and FasL in the BeWo cell line at lower concentrations. Higher concentrations appear to inhibit the production of these mRNAs. These data suggest a possible mechanism for CRH in regulating immune function during pregnancy through effects on expression of immunoregulatory retroviral proteins and FasL.

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PRE-ECLAMPSIA AFFECTS INCIDENCE OF INFANTS BORN SMALL FOR GESTATIONAL AGE DIFFERENTIALLY DEPENDING ON BOTH INFANT SEX AND DEGREE OF PREMATURITY

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Background: Preterm birth and infants born small for gestational age (SGA) are combined risk factors for neonatal mortality and morbidity. This risk of adverse outcome is greater in male than female infants. We have previously shown sexually dimorphic differences in birth weights following maternal inflammation in pregnancy. This study aimed to identify whether pre-eclampsia also exerts a sexually dimorphic effect on the birthweight of preterm infants.

Methods: Birthweight centiles of male and female infants of normotensive (n= 2276) and pre-eclamptic (n= 2907) pregnancies under 32 weeks gestation, recorded over a two year period on the ANZNN database were analysed with ANOVA and the Chi Squared statistic.

Results: Preterm infants born to mothers with pre-eclampsia had significantly higher birthweight centile than infants born following normotensive pregnancy ($p<0.001$). Females accounted for a significantly higher proportion of infants born SGA than males ($p<0.001$). Pre-eclampsia was associated with less than the expected incidence of males born SGA in very pre-term births (less than 28 weeks) (6% in males versus 11% in females). As gestational length increased (29-32 weeks) the incidence of SGA births also increased in pregnancies with pre-eclampsia, and to equivalent levels in male and female infants.

Conclusion: Early onset pre-eclampsia is a determinant of fetal growth in pre-term pregnancies. While pre-eclampsia is associated with larger birthweight centiles, there appears to be sex-specific changes to rates of infants born SGA to mothers with pre-eclampsia. In pregnancies with pre-eclampsia, lower incidences of males compared to female infants were born SGA at less than 28 weeks while increased incidences of SGA births were observed in both male and female infants born 28 to 32 weeks. This data suggests that the very pre-term male infant has a different growth trajectory in pre-eclamptic pregnancies.

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A DEVELOPMENTAL PROFILE OF THE CAPACITY OF THE INNATE IMMUNE SYSTEM TO RESPOND TO LPS FOLLOWING PRENATAL ENDOTOXIN EXPOSURE IN THE RAT

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Background: Maternal health conditions during pregnancy, such as asthma and infection, can affect immune system development and function in the offspring. Systemic infections during pregnancy are common. While infection during pregnancy is associated with

impaired immune function in early childhood its impact on the functional capacity of the immune system across the life span has not been fully characterised. This study aimed to assess innate immune function in the rodent following maternal exposure to bacterial endotoxin.

Methods: Pregnant Fischer 344 rats were exposed to either endotoxin (200ug/kg, s.c.) or saline (control; equivolume) on gestational days 16, 18 and 20. Blood cell counts, corticosterone and cytokine levels (TNFa and IL-1b) were determined in response to an *in vivo* immune challenge (Salmonella Enteritidis) in the neonatal (age 5 day), pre-weaned (19-day), pubescent (7 week), adult (3 month) and senescent (13 month) offspring.

Results: In both the neonatal and pre-weaned offspring, t he control animals exhibited significantly higher levels of cytokines and corticosterone in response to the immune challenge than the prenatal endotoxin offspring ($p<0.01$). Counts of neutrophils, monocytes and eosinophils were significantly higher in control offspring than in prenatal endotoxin offspring ($p<0.02$) in the neonatal offspring. Pubescent and adult animals from both prenatal treatment groups responded equally to the challenge, although evidence of accelerated immunosenescence was suggested.

Conclusion: This study has demonstrated a marked attenuation of the normal cytokine, corticosterone and cellular response to *in vivo* LPS stimulation in the neonatal and pre-weaned rat following prenatal exposure to endotoxin. As endotoxin mimics the immune activation observed when animals are exposed to live bacteria, our results have implications for immune development following gestational infection. While the risk of bacterial invasion and subsequent infection is high in the newborn period due to the comparative immaturity of the naïve neonatal immune system, these results suggest that prenatal infection may further compound these deficiencies.

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MOLECULAR CLONING AND CHARACTERIZATION OF BOVINE CORTICOSTEROID BINDING GLOBULIN DURING FETAL DEVELOPMENT.

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Fetal exposure to high levels of glucocorticoids during periods of chronic maternal stress, or to administered synthetic glucocorticoids during *in utero* development has clinical relevance, as there is a relationship between intrauterine development and a predisposition to the development of adult onset diseases such as cardiovascular and metabolic disorders and insulin resistance/type 2 diabetes. Corticosteroid binding-globulin (CBG) is a plasma glycoprotein that binds glucocorticoids and progesterone and can influence the proportion of the functionally active or free cortisol in circulation.

In this study, the bovine CBG gene was successfully isolated and characterized for the first time. The bovine CBG gene was found to be highly conserved when compared to ovine, human, pigs and rodents. The mRNA expression of CBG was investigated in adult, placental and bovine fetal tissues during early gestation from days 50 to 150 using semiquantitative RT-PCR and Northern blot analyses. CBG mRNA expression was detected by Northern blot analyses in adult bovine liver, and by RT-PCR in the trophoblast, allantois and yolk sac in the pre-implantation bovine conceptus, and very weakly, in fetal cotyledons from day 50 to day 150 of gestation. CBG is very highly expressed in the fetal liver at day 50, with the expression levels decreasing as bovine gestation progressed from days 100 to 150. This suggests that the need to protect the fetus against exposure to excess glucocorticoids maybe critical in early stages of development, and that the fetal liver plays a critical role through the production of CBG.

Studies to investigate and cellular localization of CBG in bovine tissue using *in situ* hybridization are currently in progress. Investigation of the role of CBG in regulating bioavailability of plasma glucocorticoid during bovine fetal development may pave the way for use of CBG as a biomarker or for the therapy of pregnancies at risk.

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Withdrawn

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PROTEIN KINASE C MEDIATES GONADOTROPIN INDUCED MITOGEN-ACTIVATED PROTEIN KINASE SIGNALLING IN EPITHELIAL OVARIAN CANCER CELL LINES

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Ovarian cancer is the eighth most common cancer in women in Australia . The gonadotropins FSH and LH have been implicated in ovarian tumorigenesis since the majority of ovarian tumours occur after menopause when serum gonadotropin levels are high . Mitogen-activated protein kinases (MAPKs) play a key role in mediating cell responses to extracellular stimuli such as hormones. The aim of this study was to identify which cell signals lead to initiation of the MAPK cascade by gonadotropins in order to understand the molecular mechanisms of gonadotropin action in ovarian tumours. In a time-dependent study, ovarian epithelial cancer cell lines (OV167, OV207, PE01, OVCAR3) were treated with FSH or LH (10 nM) and cell lysates were analysed by immunoblotting. Both hormones increased phosphorylation of ERK1/2 MAPK (extracellular signal-regulated kinase 1/2) at 5-15 min. To examine involvement of PKA signalling, cells were treated with forskolin which inhibited ERK1/2 phosphorylation in OV167, OV207 and PE01 and showed no effect in OVCAR3 cells. Forskolin strongly induced cAMP formation, whereas basal cAMP levels increased minimally in response to FSH or LH, suggesting that ERK1/2 stimulation is not linked to PKA signalling in these cells. To investigate a possible role of PKC, cells were treated with GF1090230X (PKC inhibitor) which diminished

gonadotropin induced ERK1/2 activation in all cell lines, demonstrating that PKC is involved in gonadotropin-induced MAPK phosphorylation. Treatment of OV207 with rottlerin at a specific dose for inhibition of PKC δ, abolished gonadotropin-induced ERK1/2 phosphorylation. Analysis of subcellular fractions from OV207 cells revealed translocation of PKC δ from the cytosol to the plasma membrane in response to FSH and LH, denoting activation of this novel PKC isoform. These results indicate that in epithelial ovarian cancer, gonadotropins trigger MAPK signalling through PKC δ, thus providing opportunities for therapeutic intervention in proliferative pathways in this disease.

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FOLLISTATIN, ACTIVIN A AND OTHER INFLAMMATORY PROTEINS THROUGH PARTURITION

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Objectives- Activin A and follistatin have been linked to inflammatory processes of sepsis, trauma and the acute phase response. Inflammatory systems are involved in aspects of parturition onset. This study was undertaken to investigate the relationship of follistatin with activin A, TNFα and C-reactive protein in the inflammatory processes associated with parturition in women.

Study design- Maternal venous blood was collected from at cohort of 33 women during late pregnancy (38-40 weeks) and periodically during spontaneous vaginal delivery and once within 36hours postpartum. Serum was assayed for follistatin, activin A, TNFα and C-reactive protein.

Results- Activin rose from 2.9ng/ml ±0.9 in the antenatal period to 6.1ng/ml ±1.2 in early labour (< 3cm vaginal dilation) and peaked (7.2ng/ml ±0.8) in active labour (>3cm dilation). Activin A concentrations fell (2.0ng/ml ±0.3) to below antenatal concentrations in the early postpartum period (<12 hours). Follistatin concentrations rose from an antenatal concentration of 21.0ng/ml ± 6.98 to peak at 57.9ng/ml ±5.5 in active labour and remained elevated during the early postpartum period and declined to concentrations of 26.2ng/ml ±3.9 in the late postpartum period (>12 hours). C-reactive protein remained relatively unchanged throughout the antenatal, labouring and early postpartum period (13.4mg/L ±3.9). In the late post-partum period we saw a 4-fold increase to reach 43.7mg/L ±12.0. TNFα concentrations increased from the antenatal sample (8.9pg/ml 1.4) to 18.2 pg/ml ±3.8 during early labour and remained elevated throughout the remaining sample period.

Conclusions- This study indicates that follistatin, activin and TNFa are likely to be involved in the inflammatory processes surrounding the onset of parturition. However while there is a good correlation between changes in concentration between activin and follistatin the concentrations of follistatin are in excess of the binding requirements of activin suggesting an additional role for this protein. Follistatin and activin do not appear to be directly associated with the acute phase response that activates with C reactive protein following delivery.

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P HOSPHORYLATED ANDROGEN RECEPTOR LEVELS AS A BIOMARKER IN EARLY STAGE PROSTATE CANCER

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Up to 20% of men undergoing radical prostatectomy for clinically organ-confined disease relapse with disseminated disease. It is currently not possible to predict these patients. The androgen receptor (AR) is a recognised mediator of the initiation, growth and progression of prostate cancer. Measurement of total AR in the prostate can identify non-organ confined prostate cancer. Phosphorylation of AR can be induced by androgens and may indicate AR in a biologically active form. High levels of phosphorylation of AR are known to occur at serine 81 (Ser-81) in response to androgens. Thus, it was hypothesised that measurement of phosphorylation of AR at Ser-81 in prostate tumours would better predict patient outcome following surgery than total AR levels.

In the first aim of this study the AR Ser(P)-81 antibody was characterised *in vitro*. Immunoblotting and immunocytochemical analyses revealed that Ser-81 phosphorylation was increased by androgens. In the second aim, an immunohistochemical assay for AR Ser(P)-81 was validated in paraffin embedded formalin fixed tissues to facilitate analysis of archived prostate samples. AR Ser(P)-81 and total AR immunoreactivity were then evaluated in malignant epithelial cells by image analysis in a pilot cohort of radical prostatectomy patients. No association was found between AR Ser(P)-81 and total AR immunostaining suggesting that AR Ser(P)-81 may be an improved biomarker of outcome.

A contemporary cohort of 109 patients with early stage prostate cancer were then analysed for total AR and AR Ser(P)-81. No association with rate and risk of relapse was found using Kaplan-Meier and Cox Regression analyses, respectively. These findings suggest that AR Ser(P)-81 was not a marker of disease relapse in early stage prostate cancer. Ongoing studies will determine whether it is necessary to evaluate AR Ser(P)-81 in fresh frozen tissues and whether this marker of androgen activity is more relevant in metastatic disease.

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INVESTIGATING THE ASSOCIATION BETWEEN *INHA* PROMOTER POLYMORPHISMS AND PREMATURE OVARIAN FAILURE.

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Premature ovarian failure (POF) is a common condition affecting approximately one in 100 women under the age of 40. It is characterised by amenorrhea, hypoestrogenism and elevated gonadotrophin levels. It is often an unexpected and distressing diagnosis, which coincides with infertility and menopausal symptoms. POF is a heterogeneous condition and cases are often of unknown aetiology. Genetic causes of the condition have been suggested, and variants in *INHA*, which encodes the inhibin alpha subunit, have been previously associated with POF. POF patients (n = 194) and controls (n = 162) from New Zealand and Slovenia were recruited to this study following ethical approval. POF patients and controls were screened for known polymorphisms in the *INHA* promoter (c.-16C>T, c.-124A>G and a highly polymorphic imperfect TG repeat at approximately -300bp). Genotyping was performed by restriction fragment length polymorphism (RFLP), forced RFLP and non-denaturing high performance liquid chromatography analysis. Significant differences in *INHA* promoter allele frequencies were observed between POF patient populations and controls. Significant reductions in allele frequency were observed for the -16T allele (New Zealand POF; Fisher's exact test; P = 0.029) and -124G allele (Total POF; Fisher's exact test; P = 0.048) . The TG repeat element exists as one of a number of common haplotypes of differing length (76-94bp). Significant reductions in haplotype frequency were observed for *INHA* promoter haplotypes C (New Zealand POF; Fisher's exact test; P = 0.029) and D (Slovenian POF; Fisher's exact test; P = 0.012) . We conclude that *INHA* promoter variants are associated with the development of premature ovarian failure.

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THE ROLE OF TRANSCRIPTIONAL CO-FACTORS IN THE REGULATION OF PAX8-PPAR Γ IN THYROID CELLS

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Follicular thyroid carcinomas are associated with a chromosomal translocation that fuses the thyroid-specific transcription factor paired box gene 8 (PAX8) with the nuclear receptor peroxisome proliferator-activated receptor γ (PPAR γ). We have recently shown that PAX8-PPAR γ upregulates expression of several PPAR γ -target genes including the glycerol channel, Aquaporin7 (AQP7). However, the molecular mechanism of this transcriptional function is still unknown. Transcriptional co-factors, such as PPAR γ coactivator-1 (PGC-1) and steroid receptor coactivator-1 (SRC-1), are reported to work in PPAR γ pathways. This study investigated whether these transcriptional co-factors regulate PAX8-PPAR γ function in human thyroid (Nthy-ori 3-1) cells. Co-transfected SRC-1 increased transcriptional activity of PPAR γ and PAX8-PPAR γ on the AQP7 promoter in the presence of ciglitizone. On the other hand, co-transfected PGC-1 strongly upregulated PAX8-PPAR γ transcriptional activity on the AQP7 promoter even in the absence of ciglitizone. We confirmed that these transcriptional effects depend primarily on the presence of the PPAR γ DNA binding domain (DBD), such that either mutation of the PPAR γ DBD in PAX8-PPAR γ, or mutation of the putative PPAR γ response element, was sufficient to abolish PAX8-PPAR γ -mediated regulation of the AQP7 promoter. Moreover, co-transfected PGC-1 had no effect on the PAX8-PPAR γ DBD mutant. We conclude that PAX8-PPAR γ has strong ligand-independent activity on AQP7 transcription that may be mediated via its interaction with PGC-1 and that ligand-dependent activity may be mediated via SRC-1 recruitment. In these respects, PAX8-PPAR γ transcriptional function resembles that of PPAR γ 2, and our results provide novel strategies for therapeutic targeting of these cancers.

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KISSPEPTIN CELLS PROJECT TO PRO-OPIOMELANOCORTIN (POMC) AND NEUROPEPTIDE Y (NPY) CELLS IN THE ARCUATE NUCLEUS OF THE EWE; EVIDENCE FOR TRANSMISSION OF SEX STEROID FEEDBACK TO APPETITE REGULATING CELLS

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NPY cells and POMC cells in the arcuate nucleus function respectively as orexigenic and anorexigenic systems within the brain. It is well known that the brain integrates information regarding energy balance that is transmitted to reproductive centres. Also within the brain, the function of the gonadotropin releasing hormone cells is influenced by NPY and POMC-derived peptides. Only a subset (20%) of NPY and POMC cells express steroid receptors whereas all kisspeptin cells express estrogen receptors. Kisspeptin cells are strongly implicated in the feedback effects of gonadal steroids and are also known to express leptin receptors. Both appetite regulating POMC cells and kisspeptin cells of the arcuate nucleus stimulate reproduction and are down-regulated during times of metabolic challenge. In the present study, we hypothesised that NPY and POMC cells receive input from kisspeptin cells and that the latter could transmit steroid receptor feedback information to the cells controlling energy balance. To test this hypothesis, we examined the input to POMC and NPY cells from kisspeptin cells using double-labelling immunofluorescent immunohistochemistry and Zeiss Apotome Z-stack analysis of the arcuate nucleus of 4 adult ewes. Kisspeptin-immunoreactive varicose fibers were seen in close apposition to 20% of POMC cell bodies and 11% of NPY cell bodies. These data provide the first anatomical evidence that Kisspeptin cells affect the function of cells involved in metabolic homeostasis, but the NPY and POMC cells may also provide indirect relay directly to GnRH cells. The higher level of input to POMC cells provide evidence that these cells are primary controllers or 'on/off switches' for direction of energy into either reproduction or conservation of energy. Further work will determine whether kisspeptin administration affects the function of NPY and/or POMC cells in vivo.

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LESSONS FROM A REVIEW OF THYROGLOBULIN ASSAYS IN THE MANAGEMENT OF THYROID CANCER

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Serum thyroglobulin (Tg) measurements are an increasingly integral part of ongoing management of patients with differentiated thyroid carcinoma (DTC) [1-4]. Thyroid cancer is the commonest endocrine malignancy [5]. Mortality is low, but recurrences can be frequent. Post total thyroidectomy and remnant ablation, Tg is a sensitive and specific tumour marker. Surveillance of DTC is dependent on Tg measurement, but despite this new reliance, issues regarding Tg assessment have not been appropriately addressed especially within the local context. Clinical guidelines developed overseas recommend that a Tg above 2 ug/L [6] or *any detectable level* of Tg [4] either after thyroxine withdrawal or 72 hrs after recombinant human TSH (rh-TSH) should prompt further investigation [6]. In the process of developing an institutional protocol we have identified significant clinical and technical issues regarding Tg measurement, and surprisingly found Tg is not part of an external quality control program.

We reviewed our assay at our institution and conducted a small pilot study of available assay techniques in Victoria. Our aim was to determine the potential impact on patient management when sending a blood sample to different laboratories for Tg assessment . Three laboratories measure Tg using different assay techniques. Correlation of results at the clinically significant low titres of Tg was good, but at higher levels there was divergence. There was also discrepancy in the detection of Tg antibodies which can affect Tg results.

Our small study highlights several caveats in using Tg for the ongoing management of DTC which include, caution in applying Tg cut-off values from studies conducted overseas for implementation in the local context and the variability of detecting Tg antibodies. We recommend, until there is objectivity in assessing the comparability of different assay methods, all serial Tg measurements for patients should be performed in the same laboratory using the same assay.

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THE FOLLICLE SIZE AND FLUSHING DETERMINE THE RATE OF OOCYTE RETRIEVAL

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The rate of oocyte retrieval is an important issue in the procedure of assisted reproductive technology. In current clinical practice, the process of oocyte aspiration involves follicular flushing and the usage of negative pressure, in order to increase oocyte retrieval1,2. However, it is not determined whether or not a detrimental effect of these methods on the integrity of the oocyte-cumulus complex and the remaining mural granulosa cells of the aspirated follicles might exist. The aim of this study was to investigate the benefit of follicle flushing on oocyte retrieval rates and the relationship between flushing and granulosa cell integrity. Matched size mature and immature follicles were aspirated with or without flushing and the remaining ovarian tissues were processed for histological assessment of granulosa cell layers and integrity. When flushing was not used, no oocytes were obtained from immature and mature follicles, while 42% (n=25) of the oocytes were retrieved from the flushed mature follicles only. The un-flushed mature follicles had an average number of 9.2 ± 1.8 granulosa cell layers and a cell dissociation level of 2.0 ± 0.41, whilst the flushed follicles had an average number of 6.2 ± 1.8 cells and a level of dissociation of 2.2 ± 0.41 (n=5 each group). Despite the considerable trend (>30%) in the reduction of the cell layer number, no statistical significance was found in the relationship between flushing and granulosa cell integrity. The failure of oocyte retrieval from immature follicles was not related to the oocyte size, as no significant difference in oocyte size was observed between the mature and immature follicles. In conclusion, our results support previous reports that flushing and follicle size are the major determinants of oocyte retrieval irrespective of the oocyte size. Further study is necessary to determine the effects of the usage of negative pressure on granulosa cell populations.

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RAPID *IN VIVO* EFFECTS OF ESTROGEN IN OVINE PITUITARY GONADOTROPES, LEADING TO PHOSPHORYLATION OF ERK AND CREB

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Estrogen (E) can cause rapid signalling in a variety of cell types. The aim of this study was to examine rapid effects of E in ovine pituitary gonadotropes *in vivo* and *in vitro.* In a pilot trial we treated ovariectomised (OVX) ewes with 25 m g estradiol or vehicle (i.v.) and collected pituitaries 90 min later. Using western blotting of pituitary extracts, we found that E increased ($P<0.05$) phosphorylated ERK and Akt (ser473) protein levels over levels seen with vehicle. Immunohistological analysis of the gonadotropes in these pituitaries also showed a highly significant ($P<0.001$) effect in gonadotropes. In a second study, using OVX-hypothalamo-pituitary disconnected (HPD) ewes, gonadotrope function was restored with GnRH and, in hourly pulse mode with E injection, an increase ($p<0.001$) in pERK was observed in gonadotropes within 15 min (peak response at 60 min). Peak pCREB response ($P<0.01$) occurred within 90 min. These results were obtained with both western blot and immunohistochemistry. A transient upregulation of pAkt (ser 473) occurred within 60 min of E treatment. Thus, rapid effects of E on gonadotropes involve phosphorylation of a number of second messenger proteins which may cause a rapid negative feedback effect to reduce responsiveness to GnRH. In a further series of experiments, *in vitro* treatment of pituitary cells with E (10^{-9} M) upregulated pERK and pCREB within 1 min (peak response at 15 min). *In vitro* treatment with a specific inhibitor of MAPK blocked the induction of pERK. We conclude that rapid effects of E involve phoshorylation of second messenger proteins in gonadotropes and may be the mechanism for rapid negative feedback effect of E on gonadotropin secretion. Funded by Australian Research Council.

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AN IMPROVED METHOD TO SIMULTANEOUSLY EXTRACT DNA, RNA AND PROTEIN FROM THE SAME SAMPLE

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Background: We describe a procedure for the simultaneous extraction of proteins and nucleic acid from the same experimental sample allowing for direct correlations between genetic, genomic and proteomic data. This approach, using column based kits from Qiagen (DNA/RNA kit), requires no hazardous chemicals and is a quick and simple addition to the published protocols.

Methods: Human placental samples were resuspended in buffer QRL1 and put through the RNA/DNA column kits following the manufacturer's instructions. As a control, proteins were extracted using conventional methods. Proteins were obtained from the flow-through of the columns and stored at -20^{o}C overnight with an equal volume of ethanol (100%). Proteins were washed three times in acetone and resuspended in a 2D protein extraction buffer. The profile of the proteins was then analysed by Silver staining and Western Blotting at the one-dimensional level and 2D gel electrophoresis at the 2D level.

Results: Proteins extracted from the column and solubilised in a 2D buffer were directly comparable to conventionally extracted proteins when analysed at both the 1D level and the 2D level. These proteins extracted using these methods were also compatible with Western Blotting analysis.

Conclusions: This technique provides a simple and effective way to analyse protein and nucleic acids simultaneously from a small sample size without affecting yield and quality.

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MUTATIONAL ANALYSIS OF THE SPRASA GENE IN INFERTILE COUPLES

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A new member of the c-type lysozyme/α-lactalbumin family, known as SPRASA, was recently discovered as an inner acrosomal membrane protein. SPRASA is the target of antisperm antibodies found in some infertile men[1]. These antibodies bind to SPRASA on acrosome-reacted sperm and prevent oocyte binding *in vitro*[2]. Although SPRASA is an autosomal gene, its expression to date has only been seen in the testis/sperm. The aim of this work is to investigate the function of SPRASA by the mutational analysis of the SPRASA gene sequence from couples with infertility of unknown cause.

DNA sequence analysis of the SPRASA gene from 102 (*n*= 204) infertile and 104 (*n*=208) fertile couples has identified two variants. The first variant consists of an insertion within a tri-nucleotide repeat region located immediately 5' to the translational start site. The variant identified is $(TGC)_{4\_5}$ located at c.-22TGC(4_5) (GenBank NM_173847). The minor allele was identified in 13.7% of infertile and 9.7% of fertile individuals. The second variant, c.314G>A substitution, results in a non-synonymous amino acid change at position 80 from Cysteine to Tyrosine (p.C80Y) predicted to be within the transmembrane region of the protein. This minor allele was identified in 3.2% of infertile and 1.7% fertile individuals. There was no significant difference between infertile and fertile couples in either variant.

However, one infertile individual was homozygous for the p.C80Y variant. Computer analyses of the p.C80Y variant predicted that this substitution would not be tolerated within the transmembrane region, and possibly impede the movement of the protein through the acrosomal membrane. These heterozygous variants within SPRASA appear to be tolerated within the New Zealand population. Although these variants may not result in infertility they may result in subfertility within couples. Further research is required to determine the role of SPRASA in the development of infertility. *Supported by the Marsden Fund.*

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PROTEOMIC CLASSIFICATION OF THE HUMAN WHITE BLOOD CELL RESPONSE TO GROWTH HORMONE BY PROTEIN EXPRESSION PROFILING

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The detection of human growth hormone (hGH) abuse presents unique problems. Exogenously administered recombinant hGH is virtually indistinguishable from the predominant naturally occurring isoform and is cleared from the body within 24 h. To define new biomarkers of GH administration, we have previously investigated serum proteomic profiling using surface-enhanced laser desorption/ionization time-of-flight (SELDI-TOF) mass spectrometry and have found novel putative serum biomarkers for human GH action (Chung et al, *J Clin Endocrinol Metab* 91:671, 2006). The aim of this study was to use SELDI-TOF MS to discover potential biomarkers of human GH in white blood cells (WBC). WBC extracts were collected from male recreational athletes participating in a double-blind, placebo controlled GH administration study (GH n=8, placebo n=8). Extracts from baseline and after 4 weeks' treatment were examined on protein chips with various chemical surfaces. The SELDI-TOF analysis indicated that the CM10 cation-exchange chip binds a number of GH-regulated proteins which form ionized species with m/z (mass/charge) values in the 3000-30000 range, that are either up- or down-regulated by GH. A total of 81 peaks common to the 32 profiles were identified, and peak intensities and m/z ratios were recorded. Univariate analysis of human WBC protein profiles of GH-treated subjects revealed several GH-dependent peaks which may be useful as single biomarkers of GH action. Using LC-based protein purification, peptide mass fingerprinting and MS/MS, a 10.82 kDa protein down-regulated by GH was identified as S100A8. Further validation is currently underway to discover a biomarker panel of GH responsive proteins by multivariate analysis using a binary logistic regression (GH n=23, placebo n=23). This study illustrates the novel use of human WBC proteomic profiling by SELDI-TOF MS to discover biomarkers of GH action. Supported by the World Anti-Doping Agency and the Northern Sydney & Central Coast Area Health Service.

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A RANDOMISED FOUR-WAY CROSS OVER STUDY TO COMPARE THE STEADY-STATE PHARMACOKINETICS OF TESTOSTERONE FOLLOWING APPLICATION OF DIFFERENT TESTOSTERONE METERED DOSE (MD) LOTION® FORMULATIONS AND DOSES AND ANDROGEL® IN HEALTHY MALE VOLUNTEERS WITH SUPPRESSED T

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Background : Previous work has shown transdermal testosterone systems to be effective however issues surrounding dose delivery, titration, interpersonal transfer and convenience of application remain.

Objectives: To determine the steady-state pharmacokinetics of different doses and formulations of Testosterone MD-Lotion® compared to Androgel®.

Methods: This single centre, open label study had 3 phases; screening (8 wks), treatment (5 wks) and recovery (4 wks). Sixteen healthy young men (n=7/group) were assigned to receive four treatments in random sequence for a seven day period without intervening washout. Following endogenous testosterone suppression (via Cetrorelix) groups received; (A) 3ml Testosterone MD-Lotion® (8% penetration enhancer), (B) 6ml Testosterone MD-Lotion® (8% penetration enhancer), (C) 6ml Testosterone MD-Lotion® (5% penetration enhancer) and (D) 5g Androgel. Treatments (A-C) were administered to the axilla and treatment (D) to the shoulder, abdomen and upper arm. Blood sampling and subsequent mass spectrometry analysis for total testosterone(TT), free testosterone(FT) and dihydrotestosterone(DHT) levels was undertaken prior to and at the end of each treatment period.

Results: Cetrorelix administration resulted in suppressed baseline TT, FT and DHT levels of 1nmol/L, 5pg/ml and 9ng/dl respectively. All treatments (A-D) significantly increased baseline TT (630-1180% baseline), FT (741-1610% baseline) and DHT (438-851% baseline) levels. No difference in AUC_{0-24} for any of the hormones was noted between treatments B or C, or dose normalised, baseline corrected AUC_{0-24} for treatment A and B. A significantly higher AUC_{0-24} for all hormones was found between treatments B and D.

Conclusions: There appears to be linearity in dose absorption as evidenced by the lack of significant difference between treatments A and B when adjusted for differences in volume and thereby dose. An increase in penetration enhancer from 5 to 8% does not provide increased absorption of testosterone. Treatment B provides equal or better testosterone delivery in terms of AUC_{0-24} when compared to the currently available 5g dose of Androgel.

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ROLE FOR GROWTH HORMONE IN NEURO-RESTORATION SUBSEQUENT TO FOCAL ISCHEMIA IN THE IMMATURE RAT BRAIN

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We have previously demonstrated activation of a neural growth hormone (GH) axis following hypoxic-ischemic injury (HI) to the immature rat brain. To explore a potential neuro-restorative role for GH, we examined expression of its receptor in the sub ventricular zone (SVZ) after HI in the immature rat brain, and assayed the neurogenic potential of GH on mouse neural stem cells (NSC's). Twenty-one day old rats underwent ligation of the right common carotid artery preceding exposure to 8% oxygen for 60 mins, and were sacrificed at time points ranging from 1 to 15 days post-HI. Immunofluorescence was performed using an antibody specific for the cytoplasmic tail of the GH receptor (GHR). Semi-quantitation of the level of GHR immunofluorescence using ImageJ revealed that the ratio of GHR immunofluorescence in the ipsilateral versus contralateral SVZ was elevated at 5 days after HI compared to controls (n=4 control; n=7 HI; P=0.006). Doublestaining revealed co-localisation of GHR+ cells with doublecortin in the dorsolateral SVZ and with nestin proximal to the ependyma lining the lateral ventricles. To determine the effect of GH on NSC's, mouse E15 NSC's were cultured for three days with rat GH, as well as 1 μM BrdU for the last 48 hours. GH treatment robustly increased the number of BrdU+ NSC's by up to 350% compared to vehicle-treated cultures (P<0.01). These findings implicate a role for GH in injury-induced neurogenesis and suggest that long term GH treatment may be neuro-restorative to the injured juvenile brain.

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TESTOSTERONE STIMULATES EXTRA-HEPATIC BUT NOT HEPATIC FAT OXIDATION AT SYSTEMIC REPLACEMENT DOSES IN HYPOPITUITARY MEN

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Fat is oxidised in the liver and in extra-hepatic tissues. Oral estrogen suppresses hepatic fat oxidation (Fox) through a first pass effect. As testosterone (T) enhances whole body Fox and resting energy expenditure (REE) in hypopituitary men (Gibney et al, JCEM 2005, 289), we aimed to determine whether this arises primarily from the liver by comparing the metabolic impact of T administered via oral route (using doses designed to achieve physiological concentrations in portal blood) compared with a standard transdermal T replacement dose. Twelve hypopituitary men (age 53.1 ± 4.1 y, mean ± SEM) with GH and T deficiency participated in an open-label cross over study of 2 wk treatments of transdermal T (tdT 5 mg), followed by 2 wk washout period, and stepwise incremental doses of oral crystalline T (10, 20, 40, and 80 mg) in the absence of GH replacement. Serum T, IGF-I, metabolic effects (REE, Fox) and SHBG as a marker of excessive hepatic androgen exposure measured at the end of each treatment period were analysed by repeated measures ANOVA with significance ($p<0.01$) determined after Bonferroni's correction.

	tdT 5 mg	Washout	oT 10 mg	oT 20 mg	oT 40 mg	oT 80 mg
T nmol/l	16.6 ± 3.1 †	4 ± 0.8	4 ± 0.9	4.6 ± 1.4	4.5 ± 0.9	6.2 ± 1.3
SHBG nmol/l	27.6 ± 3.6	32 ± 4.1	30.5 ± 4.5	31.3 ± 4.8	29.8 ± 3.9	23.9± 4.2*^
Fox mg/min	60.3 ± 5.7*	46.4± 5.7	49.1± 5.8	40.6 ± 4.2	50.9 ± 5.8	46.8 ± 6.1

$P<0.01$ * vs. washout; ^ vs. oT 40 mg; † vs. all other treatment groups. Mean blood T levels were not increased by 10, 20, 40 or 80 mg oral T, but was in the physiological range during transdermal delivery. Blood SHBG was unaffected by 10, 20, 40 mg oral and transdermal T but fell significantly with 80 mg oral T. No T treatment changed plasma IGF-I or REE. Fox increased significantly with transdermal but not with any dose of oral T. In summary, oral T at doses sufficient to induce pharmacological hepatic androgenic effects without increasing systemic T concentration, had no effect on Fox, which was however stimulated by transdermal T at a standard replacement dose. In conclusion, T does not stimulate hepatic Fox but enhances whole body Fox by acting on extrahepatic tissues in the absence of GH replacement in hypopituitary men. (Supported by the NHMRC of Australia; Maynepharma provided Androderm)

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GROWTH HORMONE AND TESTOSTERONE EXERT DIFFERENTIAL AND ADDITIVE EFFECTS ON LEAN BODY MASS IN RECREATIONAL ATHLETES

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GH and testosterone (T) are anabolic agents used to enhance sporting performance. As both hormones act through different mechanisms, it is likely that their effects on body composition, especially on components of lean body mass (LBM) such as extracellular water (ECW), may not be the same. Whether men and women respond equally to GH is unclear. The aims of this study were to compare (i) the effects of GH, T and in combination on body composition, and (ii) gender difference in response to GH. 97 recreational athletes, aged 27.9 ± 0.6 years, participated in this placebo-controlled prospective double blind study. 64 men were randomised to 8 wk treatment with placebo, GH (2 mg/d), T (Sustanon 250 IM mg/wk), or GH+T; and 33 women were randomised to placebo or GH. Body composition was measured by DXA and ECW was estimated using the bromide dilution technique. Statistical analysis was performed using one-way ANOVA with post hoc significance determined after Bonferroni's correction. Data are presented as change from baseline and expressed as mean ± SEM.

	Men				Women	
	Placebo	GH	T	GH+T	Placebo	GH
Δ FM kg	-1.1 ± 0.5	-1.8 ± 0.3	-1 ± 0.7	-2.1 ± 0.5	-0.1 ± 0.3	-2.4 ± 0.4 *
Δ LBM kg	0.8 ± 0.3 †	3.5 ± 0.5 *†	3.2 ± 0.4 *†	6.6 ± 0.6 *	0.5 ± 0.3	2.8 ± 0.5 *
Δ ECW l	-0.3 ± 0.6 †	2.1 ± 0.5 *	1.0 ± 0.7 †	3.3 ± 0.7 *	0.1 ± 0.4	1.2 ± 0.5

$p<0.01$ * vs. placebo; † vs. GH+T. In men, GH treatment significantly increased both LBM and ECW, while T increased LBM only compared to placebo. The change in ECW with GH was twice that of T, however the difference did not reach statistical significance. GH+T increased LBM more than with either treatment alone, while the increase in ECW was greater compared to T but not GH alone. In women, GH significantly reduced FM, and increased LBM but not ECW. These changes were not significantly different from those in men. In summary, both GH and T increased LBM but only GH increased ECW. Combined treatment enhanced LBM without significantly changing the ECW component attributable to GH. The effects of GH on LBM were not different between men and women. We conclude that GH and T exert differential and additive effects on LBM. As the non-ECW-component of LBM is body cell mass, T exerts a greater protein and cellular anabolic effect than GH. Funding support from the World Anti-Doping Agency and Australian Government Anti-Doping Research Program. Novo Nordisk provided GH, and Organon provided testosterone.

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EFFECTS OF 11-KETOTESTOSTERONE ON HEPATIC PHYSIOLOGY OF THE SHORTFINNED EEL, ANGUILLA AUSTRALIS, IN VIVO

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Freshwater eels undergo a yellow-to-silver transformation (silvering metamorphosis) in preparation for a long-distance spawning migration. This event is characterised by numerous morphological and physiological changes that can be induced, at least in part, by the androgen 11-ketotestosterone (11-KT). Notably, changes in liver function during silvering have been documented, but the effects of 11-KT on liver physiology remain unknown. To test the hypothesis that androgens mediate the changes in liver function during metamorphosis, eels were implanted with slow-release pellets with or without 2.5 mg 11-KT. Following two weeks of starvation, eels were euthanased for tissue collection. In addition, pre- ('yellow') and post-metamorphic ('silver') eels were sampled from the wild. Livers were removed, weighed, examined by microscopy and analysed for lipid content and relative mRNA levels of lipoprotein lipase (LPL) and insulin-like growth factor-I (IGF-I; sum of both splice variants). Relative liver weight, but not neutral lipid content, was significantly higher in fish treated with 11-KT than in controls. Histologically, hepatocytes from steroid-treated fish appeared larger due to increased vacuolisation, resembling the observations on livers from silver eels. Furthermore, silver eels showed significantly elevated LPL mRNA levels compared to LPL transcript abundance in yellow eels, but no such effect was apparent following 11-KT treatment. Unexpectedly, IGF-I mRNA levels (primarily long splicing variant) increased over three-fold in 11-KT treated fish compared to controls, an effect opposite to that seen during silvering. Our findings implicate 11-KT in mediating some of the morphometric and microscopical hepatic changes occurring during the silvering metamorphosis. However, changes in molecular targets in response to androgen treatment were unlike those seen during silvering; this is possibly due to differences in feeding status between animal groups, feed intake most likely being much higher in yellow than in silver eels or fish in the experimental (starving) groups.

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ERRGAMMA, AN ORPHAN NR REGULATOR OF METABOLIC GENE EXPRESSION IN SKELETAL MUSCLE CELLS.

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Estrogen-related receptors (ERRs) are orphan members of the nuclear receptor superfamily (NR). In particular, ERRα and ERRγ are abundantly expressed in tissues involved in oxidative metabolism and rich in mitochondria such as the heart, skeletal muscle, brown adipose tissue. Studies have shown ERRα modulates adiposity and metabolism (1). It is still unclear whether ERRγ plays a major role in the regulation of lipid and energy homeostasis in the major mass peripheral metabolic tissue, however, ERRγ has an important role in modulating ERRα gene expression (2). Natural ligands for ERRs have not been identified to date, however, several synthetic compounds activating or repressing ERR activities have been described (3,4). In this study, we utilise these agonists GSK4716 (phenolic acyl hydrazones), antagonist diethylstilbestrol and ERRγ-siRNAs in an in vitro skeletal muscle cell culture model to elucidate the role of ERRγ. Skeletal muscle is the most metabolically demanding major mass metabolic tissue that relies heavily on fatty acids as energy source. We observed changes in the expression of key regulators of lipid, glucose and energy homeostasis, such as PGC-1α following treatment with ERRγ agonist/antagonist. Moreover, these changes were concordant with several changes in metabolism.

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ADIPONECTIN ELEVATES $[CA^{2+}]I$ IN RAT SOMATOTROPES THROUGH ACTIVATION OF ADIPONECTIN RECEPTORS LEADING TO GROWTH HORMONE SECRETION IN VITRO

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Adiponectin is a cytokine released by adipocytes and has been demonstrated as an insulin sensitizing hormone, regulating metabolic balances. Growth hormone (GH) is an important anabolic hormone from pituitary gland and the secretion is under the influence of metabolic regulatory hormones, such as leptin and orexin. It is an open question whether adiponectin has any effect on somatotropes or regulates the level of GH. Based on functional crossover of adiponectin and GH on maintaining normal metabolic balance, it is proposed that adiponectin may act on somatotropes in vitro. In this report, we demonstrated the presence of adiponectin receptors 1 and 2 in pituitary cells including somatotropes by western blot analysis and immunocytochemistry using adiponectin receptor antibodies, and RT-PCR for receptor expression. Administration of adiponectin (2ug/ml) directly onto somatotropes induced an immediate increase in $[Ca^{2+}]i$ indicated by Fluo-3 fluorescence intensity under confocal microscope. This increase in $[Ca^{2+}]i$ was further dissected using thapsigargin (depleting the InsP3-sensitive Ca^{2+} pool) and Ca^{2+}-free bath solution (abolishing the Ca^{2+}-influx through channels) and demonstrated to be a composite of both Ca^{2+} release from the InsP3-sensitive pools and the Ca^{2+} influx through membrane Ca^{2+} channels. Signaling systems were also dissected for the involvement of PLC (U73122), PKA (H89, KT5720)

and PKC (chelerythrine, calphostin C and GO6978). It has been shown that PLC was involved in Ca^{2+}-release whereas PKC mediated the Ca^{2+}-influx through Ca^{2+} channels. Functionally, adiponectin increased GH secretion from cultured somatotropes, which was blocked by L-type Ca^{2+} channel blocker (nifedipine). In conclusion, adiponectin as a circulating hormone may elevate GH levels through activation of adiponectin receptors and increase in $[Ca^{2+}]i$ in somatotropes.

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CHARACTERISATION OF THE N/C-INTERACTION IN THE MINERALOCORTICOID RECEPTOR

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The mineralocorticoid receptor (MR) regulates various physiological processes including the regulation of electrolyte homeostasis, and blood pressure. Clinical studies underscore the efficacy of MR blockade in the treatment of cardiovascular diseases. However, this is limited by the potential side effects. Despite the apparent clinical significance of the MR as a therapeutic target, an understanding of the molecular mechanism underlying signal transduction by the MR is not completely understood. Recent studies have identified an interaction between the N-terminal domain and the C-terminal, ligand-binding domain of the MR (N/C-interaction) that occurs in response to aldosterone[1]. In this study we sought to fully characterise the N/C-interaction in the MR.

The mammlian-2-hybrid (M2H) assay is being utilised to assess: i) a range of natural and synthetic MR ligands; ii) the role of the promoter in the M2H assay and iii) the response in different cell lines. GST pull-down assay has been used to establish whether a direct protein-protein interaction is involved.

In contrast to aldosterone, which strongly induces the interaction, the physiological ligands deoxycorticosterone (DOC) and cortisol weakly promote the interaction but predominantly inhibit the aldosterone mediated N/C-interaction. Similarly, progesterone and dexamethasone antagonise the interaction. In contrast, the synthetic agonist 9 a fludrocortisol robustly induces the interaction. These studies in COS-1 cells are currently being repeated in different cells lines and with different promoters. The GST pull-down assay demonstrates a direct aldosterone dependent protein-protein interaction.

The ability of the N/C-interaction to discriminate between MR agonists suggests a conformational difference in the ligand-binding domain induced by these agonists. The characterisation of the N/C-interaction in MR may provide a valuable platform for the development of MR modulators for use as therapeutic agents in the treatment of cardiovascular diseases.

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SHIFTWORK SIMULATION IN RATS IMPAIRS GLUCOSE TOLERANCE AND INSULIN SECRETION AND SENSITIVITY

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Shiftwork is increasing, with more than 14% of Australians working outside the "normal" working hours. While it is widely acknowledged that shiftwork induces fatigue with major occupational health and safety consequences, it is now clear that shiftwork also substantially increases the risk of developing metabolic syndrome. Nevertheless, we lack understanding as to why this occurs and the basis for developing effective interventions. Disruption of clock gene transcription factor expression and action through continuous shifting of the light/dark cycle may alter metabolic rhythms, impair insulin secretion and responsiveness and lead to the development of impaired glucose tolerance. To test this hypothesis, male rats (6 wk) were exposed to a simulated shiftwork paradigm mimicking 3 twelve hour night shifts per week for 28 days. Control groups remained on a normal 12L:12D photoperiod. IPGTT were conducted 1 day, 1, 2 and 3 weeks after the last shift, while a hyperinsulinaemic euglycaemic clamp (HEC) was conducted 1 day after the last shift to assess insulin sensitivity. At the end of shiftwork and resumption of the normal light/dark cycle, glucose tolerance was significantly impaired, which persisted for at least 2 weeks, finally normalising after 3 weeks. Insulin secretion in response to glucose injection was also substantially impaired, which persisted for at least a week after cessation of shiftwork. At one day after the cessation of the shiftwork simulation, insulin sensitivity was significantly reduced by 46% in rats previously exposed to shiftwork compared to controls. Exposure of young adult rats to "shiftwork" substantially impaired glucose tolerance and insulin secretion and induced insulin resistance, which persisted for up to 2 weeks. This study provides compelling evidence that alterations in lighting and subsequent rhythm disruption in humans may contribute to the development of metabolic syndrome in susceptible people working outside normal working hours.

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THE ROLE OF INFERIOR PETROSAL SINUS SAMPLING FOR ACTH- DEPENDENT CUSHING'S SYNDROME.

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Objective: Inferior petrosal sinus sampling (IPSS) with CRH stimulation is considered the gold standard for the differential diagnosis of ACTH-dependent Cushing's syndrome. The aim of this study is to evaluate its role in confirmation and lateralisation of a pituitary source in suspected Cushing's disease (CD).

Methodology: Nine patients with CD with indeterminate MRI underwent IPSS from August 2004 to February 2007. Three were being assessed for recurrent disease. The following parameters were evaluated: (i) Central- to- Peripheral ACTH gradient for localisation, (ii) Inter-petrosal sinus gradients for lateralisation, (iii) Surgical histopathology, and (iv) Post-operative clinical course.

Results: A Central- to- Peripheral ACTH gradient of ≥ 2.0 was found in 7/9 patients at base line and a gradient of ≥ 3.0 in all patients after CRH stimulation, confirming pituitary source of ACTH excess. Inter-petrosal sinus gradient of ≥ 1.4 was observed in 8/9 patients at base line and in all patients after CRH stimulation. This correlated with lateralisation of the excess ACTH source in all patients undergoing their first resection resulting in remission.

For recurrent CD patients, the site of resection was made intra-operatively based on macroscopic appearance and frozen section. The IPSS lateralisation did not appear to have influenced the decision. Two had pituitary adenoma confirmed, however all three did not achieve remission.

Conclusion: IPSS can help confirm the location and even lateralise the source of ACTH production. In patients undergoing their first resection, the results are useful to direct surgeons to the appropriate site of excision (100% sensitivity & specificity). For patients with recurrent disease, IPSS was useful in confirming the source of excess ACTH. However, its role in determining and guiding the site of surgical excision still warrants further investigation.

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(2) Machado MC et al, The role of desmopressin in bilateral and simultaneous inferior petrosal sinus sampling for differential diagnosis of ACTH-dependant Cushing's syndrome.

(3) Lynnette K et al, Evaluation and treatment of Cushing's syndrome, The American journal of Medicine, vol118(12), 2005

(4) Yutaka Kai et al, Usefulness of multiple-site venous sampling in the treatment of ACTH-producing pituitary adenomas; Surgical Neurology, Volume 59(4), 2003.

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LINOLEIC ACID INDUCES AN INCREASE IN INTRACELLULAR CALCIUM CONCENTRATION AND MEMBRANE HYPERPOLARIZATION OF PRIMARY CULTURED RAT PANCREATIC B-CELLS

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Free fatty acids (FFAs) activate their membrane receptor, GPR40, and GPR40 activation increases intracellular Ca^{2+} concentration ($[Ca^{2+}]i$), which leads to insulin secretion (1). Although reduction of voltage-dependent potassium currents was proposed to participate in this process (2), the mechanism of the increase in $[Ca^{2+}]i$ induced by FFAs is not fully understood. The $[Ca^{2+}]i$ level and insulin secretion are regulated by electrophysiological activities of β-cells. In this study, we observed the effects of linoleic acid (LA) on $[Ca^{2+}]i$ and membrane potential in primary cultured rat pancreatic β-cells. LA (20 m M) induced a biphasic increase in $[Ca^{2+}]i$ under 3 mM glucose condition in β-cells. LA-induced increase in $[Ca^{2+}]i$ was composed of an immediate transient increase for about 2 min and a delayed long-lasting one that sustained for more than 10 min. The first transient increase was eliminated by thapsigargin pretreatment of the cells, but not blocked by removal of extracellular Ca^{2+}. The second delayed increase was not totally removed by both thapsigargin pretreatment and removal of extracellular Ca^{2+}. Simultaneously with $[Ca^{2+}]i$ increase, membrane potential of β-cells was significantly hyperpolarized by LA. LA-induced hyperpolarization of β-cells was totally abolished by blockade of K_{ATP} channels with tolbutamide. K_{ATP} currents recorded by patch clamp were significantly increased by LA. In conclusion, LA-induced increase in $[Ca^{2+}]i$ in rat β-cells is mainly due to Ca^{2+} release from intracellular Ca^{2+} stores and partially contributed by Ca^{2+} influx. LA induces β-cell hyperpolarization by activating K_{ATP} channels. The increase in $[Ca^{2+}]i$ leads to insulin secretion, but the hyperpolarization may prevent glucose-stimulated insulin secretion (GSIS). The results support that FFAs may on one hand compensate insulin secretion, and on the other hand evoke the defect in GSIS in the development of diabetes.

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(2) Feng DD et al, Endocrinology 2006; 147: 674

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EFFICACY AND SAFETY OF LANREOTIDE AUTOGEL IN PATIENTS WITH ACROMEGALY PREVIOUSLY TREATED WITH OCTREOTIDE LAR

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Long-acting somatostatin analogs are effective treatment for acromegaly. Two agents (Octreotide LAR and Lanreotide Autogel) are available in Australia . Few studies have examined their comparative efficacy or dose equivalence. In this open-label 'switch study' eight patients (4 female) with active acromegaly previously treated with Octreotide LAR were transferred to Lanreotide treatment for a period of 40 weeks. All had IGF-1 levels in the age-specific normal range while on a stable dose of Octreotide LAR (10, 20 or 30mg monthly) for at least 4 months prior to entering the study. All patients were switched to Lanreotide Autogel 90 mg every 4 weeks by deep s.c. injection from week 0 to week 12. The dose was then titrated to 60,90 or 120 mg, depending on IGF-1 levels.

The main study endpoints were IGF-1 and GH levels, dosage requirements, safety, patient tolerability and quality of life (QoL). IGF-1 levels were in the normal range in all patients at week 0, and all remained normal at Week 44. All patients had GH levels < 5.0 mU/L at Week 0, but 2 of 8 had GH levels >5 mU/L at Week 44 (the elevated levels were 5.3 and 6.1 mU/L). Despite this there was no statistical difference in paired GH results from baseline to conclusion. After dose titration 4 patients ended up with a lower dose of Lanreotide than expected for their starting dose of Octreotide, 1 higher, 3 equivalent. QoL was measured using an adapted version of the SF-36 tool. Scores of physical health at Week 0 and Week 44 were stable in 6 of the 8 patients and improved in 2. Mental health scores were stable in 5 of the 8 patients and improved in 3. The main reports of improvement related to less pain at injection sites. Some patients opted to undertake home injection of Lanreotide at the end of the study, in order to reduce the frequency of clinic

visits. Six adverse events were thought to be related to Lanreotide - 5 of these were pain or lump at site of subcutaneous injection, 1 case of diarrhoea following injection.

In patients with proven therapeutic responses to Octreotide, Lanreotide treatment was similarly effective in maintaining control of IGF-1 levels. However, GH levels rose modestly in two patients despite similar IGF-1 control. The reason for this discrepancy, and its clinical significance, is unclear. When IGF-1 levels are used for dose titration a smaller equivalent dose might be achieved using Lanreotide, with possible cost savings.

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INFECTIOUS CAUSES OF OF ADRENAL INSUFFICIENCY - PREVALENCE, CLINICAL FEATURES AND LONG TERM FOLLOW-UP IN A SOUTH INDIAN TERTIARY HOSPITAL.

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Background: Adrenal tuberculosis (TB) and histoplasmosis are significant causes of Addison's disease (AD) in India. However, the discriminatory features, optimal management and natural history of these conditions is not well-documented.

Aim: To determine the aetiologies of AD in an Indian tertiary institution, identify clinical features associated with adrenal infections and describe their long term course.

Method: Patients presenting with a new or existing diagnosis of AD between 2001-2007 were identified. Patients with infectious adrenal lesions without overt AD were analysed separately. Demographics, clinical presentation, biochemistry, imaging, histopathology, treatment and follow-up were recorded.

Results: Of 45 patients with AD, 29% were of infectious aetiology (11.1 % histoplasmosis, 8.8% confirmed TB, 4.4 % presumed TB, 4.4% unclassified granulomatous), 9% had other definable causes (lymphoma, infarction, adrenoleukodystrophy) and 62% were labeled 'autoimmune/idiopathic'. Patients with infectious versus idiopathic AD were more likely to be male (92 % vs 48% p=0.01), older (average age at diagnosis 46 versus 33 years, p<0.01), with higher creatinine at presentation (median 1.5 vs 1.0 p<0.01) and lower albumin:protein ratio. Presence of fever, leukocytosis or raised ESR was not discriminatory. Pathological and radiological features of 17 patients with infectious adrenal lesions, with or without insufficiency were documented. Follow-up ranged from 0 to 58 months. 6 out of 7 patients who had repeat imaging performed between 4 to 58 months post diagnosis had persistent lesions despite treatment, with minimal or no shrinkage. 1 patient rebiopsied at 45 months had persistent histoplasma. There were no cases where steroid or anti-fungal therapy were ceased, even up to 5 yrs from diagnosis.

Summary: Adrenal infections should be considered in persons from India presenting with AD. Lesions may persist for years despite antibiotic therapy; optimal treatment duration and potential for hormonal recovery remains unclear. Re-biopsy of adrenal lesions and reassessment of adrenal sufficiency will reveal important new information.

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LUGOL'S IODINE IN PREPARATION FOR THYROIDECTOMY

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Introduction: Lugol's iodine had been available since 1829. Today, its clinical uses in hospitals are primarily for thyroid protection in patients with hepatoma undergoing postoperative I^{131}-labelled lipiodol therapy, and rapid correction of hyperthyroidism in selected patients pre-thyroidectomy. The aim of this study was to describe its pattern of use and to assess its effectiveness in preparation for thyroidectomy.

Methods: We performed a retrospective review of all patients prescribed Lugol's iodine pre-thyroidectomy at Westmead Hospital, Sydney between October 1999 and December 2006. Patients were identified using hospital pharmacy dispensing records. Patient medical records were individually reviewed and thyroid function tests prior to Lugol's iodine use and immediately pre-thyroidectomy were recorded.

Results: Between October 1999 and December 2006, 26 patients were identified. Of these, 8 patients were prescribed Lugol's iodine in preparation for thyroidectomy after failure to respond or contraindication to thionamides. Seven patients were diagnosed with Grave's disease, while 1 patient had lymphocytic thyroiditis. Prior to the initiation of Lugol's iodine, all 8 patients had suppressed TSH (<0.1 prior to 2004, <0.04 after 2004), with a mean (+/- SD) free T4 of 37.7 +/- 10.3 pmol/L, and a mean free T3 of 16.4 +/- 5.9 pmol/L. Patients received varying doses and frequency of Lugol's iodine, ranging from 2 drops bd for 24 hours to 1ml qid for 9 days pre-operatively. While their immediately pre-operative TSH remained suppressed, their mean free T4 was significantly lower at 15.0 +/- 10.9 pmol/L (p=0.002), and mean free T3 also significantly lower at 4.2 +/- 1.1 pmol/L (p=0.001).

Conclusion: In a hospital setting, the majority of Lugol's iodine was prescribed for thyroid protection in patients receiving treatment for hepatoma. In patients receiving it prior to thyroidectomy, the majority had Grave's disease. Lugol's iodine inhibits thyroid hormone release and reduces thyroid gland vascularity acutely, and is effective in lowering both free T4 and free T3 pre-thyroidectomy.

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LARGE SECRETORY GANGLIONEUROMA IN A 19 YEAR OLD MAN.

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Background: Ganglioneuromas are tumours of the sympathetic system and together with ganglioneuroblastomas and neuroblastomas are collectively known as neuroblastic tumours. Ganglioneuromas are rare, benign, fully differentiated tumors that contain mature Schwann cells, ganglion cells, fibrous tissue, and nerve fibers. This lesion can grow almost anywhere along the paravertebral sympathetic ganglia and sometimes in the adrenal medulla. These tumours tend to occur in children usually 5 to 7 years of age and are considered to be benign and usually not secretory.

Case Presentation: We report a 19 y.o. male patient with a past history of primary hypothyroidism who presented with shortness of breath, hypotension and tachycardia. A CT pulmonary angiogram showed bilateral pulmonary emboli but a 15cm right adrenal mass compressing the inferior vena cava was noted as an incidental finding. He was thrombolysed and started on clexane subcutaneously. Further investigations revealed three 24hr urinary catecholamines that were within normal range , 24hr urinary free cortisol was 138 (50-350 nmol/24h), 24hr urinary homovanillic acid 89 (<44 umol/L) , 24hr urinary dopamine excretion on first collection was 26.07 (<3.50 umol/24h) and on the second collection was 19.27.

MIBG scan showed increased uptake in the right adrenal region but no other abnormal uptake. He underwent laparoscopic adrenalectomy 2 months following diagnosis.

Histology showed benign ganglioneuroma weighing 762 grams arising from the right sympathetic chain and a normal appearing right adrenal gland separated from the ganglioneuroma. Repeat 24 hr dopamine excretion returned to normal (2.11 umol/24h).

Conclusion: This is a patient with a large secretory ganglioneuroma with increased urinary homovanillic acid, increased urinary dopamine excretion and a positive MIBG scan. This case illustrates that although ganglioneuromas are benign, they can be functionally active and should be considered as a rare cause of adrenal masses.

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ANNUAL ZOLEDRONIC ACID AS ANTI-RESORPTIVE THERAPY FOR OSTEOPOROSIS IN CLINICAL PRACTICE

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Introduction : Zoledronic acid (ZA) has been reported in controlled clinical trials to improve bone mineral density[1] (BMD) and reduce fracture risk[2] in post-menopausal osteoporosis. We describe our clinical experience with ZA in patients with osteoporosis intolerant of, or with contra-indications to, oral anti-resorptive agents.

Methodology : This was an observational retrospective audit of the patients of two endocrinologists (CHR, GIH), based on clinical assessment and chart review. The aims were to review the (i) tolerability/safety and (ii) efficacy of ZA in this group of patients as assessed by (i) occurrence of adverse effects and (ii) occurrence of symptomatic new fractures (confirmed radiologically), serial bone densitometry and biochemical measurement of markers of bone turnover. Patients were included if they had received at least two doses of ZA (4mg iv annually) and regular clinical and biochemical monitoring, as well as at least one follow-up DEXA.

Results : 46 patients (10 males) fulfilled the inclusion criteria. No patients developed jaw osteonecrosis, although one experienced delayed healing after tooth extraction. Seven developed minor adverse effects, and one eyelid/eyebrow irritability. Ten patients experienced minimal trauma fractures during follow-up.

The baseline femoral neck T-scores ranged from –1.00 to –5.59 with a mean of –2.62.

In the lumbar spine, BMD increased from baseline after 1-2 years in 35 patients and declined in 7; mean change was 2.77% per annum. Similarly, in the femoral neck, BMD increased in 21 patients and declined in 23; mean change was 0.61% per annum.

Data regarding patient characteristics, biochemical results, frequency of atrial fibrillation and BMD data at 3-4 years will be presented at the meeting.

Conclusion : ZA had variable efficacy and was generally well-tolerated in this heterogeneous group of high-risk patients in whom oral bisphosphonates were deemed unsuitable.

(1) Reid et al. NEJM 2002;346(9):653-61
(2) Black et al. NEJM 2007;356(18):1809-1822

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URINARY IODINE CONCENTRATIONS IN EARLY PREGNANCY.

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Iodine is an essential element for fetal neurological development. Fetal pituitary TSH is detectable by 8-10 weeks' gestation, with significantly increased fetal pituitary-thyroid axis activity between 16 and 22 weeks, coincident with development of the primary plexus of the pituitary portal vascular system and of continuity between the primary and secondary plexuses, allowing delivery of TRH to the anterior pituitary.

South-eastern Australia is a region of endemic iodine deficiency with sources of dietary iodine limited to dairy produce, seafood and iodised salt. The replacement of iodophors as disinfectants in the dairy industry has resulted in a significant reduction in the iodine

content of milk (1). Mild iodine deficiency was shown in women in early 3rd trimester pregnancies during 2004 (2) highlighting the need to ensure adequate iodine intake.

Since fetal neurological development commences early in gestation, we wished to determine maternal iodine status in first trimester pregnancies. Maternal urine samples were obtained at the first antenatal visit for consecutive women attending 2 private obstetricians. Maternal age and gestation were recorded and urine samples analysed for iodine concentration (UIC) at The Institute of Clinical Pathology and Medical Research, Westmead. UIC was classified by WHO/International Council for the Control of Iodine Deficiency Disorders criteria (<20µg/L: severe iodine deficiency, 20-49: moderate, 50-99: mild, ≥100: normal). Subjects found to have low UIC were prescribed supplements.

Mean (±SD) maternal age was 34.5±4.0 yr (median 34.5 yr) at a mean (±SE) gestation of 9.9±0.8 weeks (median 10 weeks, n=35). Mean UIC was 99.3±11.4µg/L, median 80.0 with interquartile range 85. 25.7% of samples were <50µg/L (cf 11% of 123 privately-insured patients reported by Travers et al. [2]; chi-square 4.03, $p<0.05$). Two samples were <20µg/L. There was no correlation between UIC and maternal age or gestation.

These data concur with others (2) but in particular highlight the need to assess maternal iodine status in early pregnancy in order to limit any deleterious effect of maternal iodine deficiency on fetal neurological development. Data collection is continuing and will be presented.

(1) Li M et al. Med J Aust (2006) 184:307.
(2) Travers C et al. Med J Aust (2006) 617-20.

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A CASE OF HYPERCALCEMIA, ADRENAL INSUFFICIENCY AND THYROTOXICOSIS

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A 50-year-old woman with a past history of hypothyroidism on thyroxine replacement presented with a 4-month history of lethargy, nausea and weight loss . Symptoms were originally attributed to depression, and she was referred to hospital to with suspected anorexia nervosa. On admission, she was confused with generalized melanotic pigmentation, volume depletion, and wasting, but no other physical findings to assist diagnosis. The plasma calcium was 3.41mmol/L, phosphate 1.03mmol/L and PTH suppressed. Further investigations revealed hyperthyroidism (serum TSH < 0.004, free T4 32.7mmol/L) and adrenal insufficiency (basal cortisol 64).Thyroid and adrenal antibodies were negative.

The initial management of hypercalcemia involved vigorous intravenous fluid therapy and frusemide without improvement Glucocorticoids were added with subsequent normalization of calcium levels. Thyroid function improved rapidly after ceasing thyroxine. The admission was complicated by infection, anaemia, and electrolyte imbalance. She made a full recovery, but failed to attend follow-up review. The diagnosis of polyendocrine syndrome type 2 was made after exclusion of other causes.

Hypercalcemia is uncommonly associated with adrenal insufficiency, although the exact mechanism is unclear. Proposed mechanisms include impaired calciuresis and increased bone resorption. . Bone resorption is partly mediated by thyroid hormone, via a process normally inhibited by glucocorticoids. Hypercalcemia commonly occurs in hyperthyroidism, the major mechanism being increased bone resorption mediated by thyroid hormone.

Case reports suggest that adrenal crisis may be precipitated by thyroxine therapy in patients with known hypothyroidism and previously undiagnosed adrenal insufficiency (polyendocrine syndrome type 2). Presumably this results from the increased hepatic metabolism of cortisol in the presence of thyroxine

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MEASUREMENT OF GLUCAGON-LIKE PEPTIDE 1 (GLP-1) LEVELS IN NONINSULINOMA PANCREATOGENOUS HYPOGLYCAEMIA SYNDROME (NIPHS)

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Background: NIPHS is a rare cause of endogenous insulin mediated hypoglycaemia characterised by the histological appearance of nesidioblastosis. The pathogenesis of the syndrome is unknown. There have been recent case reports linking gastric bypass surgery for the treatment of obesity and the development of nesidioblastosis. GLP-1 becomes elevated following bypass surgery and is thought to play an important role in the weight loss and reversal of diabetes following successful surgery, and has been postulated to play a role in the development of nesidioblastosis in bypass patients. GLP-1 has not been measured in patients with sporadic NIPHS. We sought to investigate whether there might be an exaggerated GLP-1 response to a mixed meal in a patient with NIPHS.

Clinical Case: A 71 year old lady was referred for investigation of hypoglycaemia. There was a 20 year history of neuroglycopaenic symptoms. Testing confirmed insulin mediated hypoglycaemia: random BSL 1.6mmol/L (NR 3.6-6.0), insulin 105.1 mU/L (0-20). A 3 day fast produced hypoglycaemia with inappropriately elevated insulin and C-peptide levels. Sulphonylurea screen was negative. A CT demonstrated a mass within the tail of the pancreas consistent with an islet cell tumour. A laparoscopic distal pancreatectomy was performed. Pathology demonstrated widespread nesidioblastosis as well as an insulinoma measuring 10mm. Her postoperative course was complicated by a pancreatic leak which was treated conservatively. Insulin was only required peri-operatively. She has been followed for 2 years and remains free of hypoglycaemic symptoms. Follow-up oral glucose tolerance tests have been normal. A recent CT demonstrates a new pancreatic mass. MEN1 gene testing is negative.

Results: GLP-1, BSL and Insulin levels obtained following the mixed meal are shown in the table.

SUBJECT	GLP-1 (pmol/L) Fasting	GLP-1 (pmol/L) Peak	BSL (mmol/L) Fasting	BSL (mmol/L) Peak	INSULIN (mIU/L) Fasting	INSULIN (mIU/L) Peak
PATIENT	4.7	12.4 (30mins)	5.6	8.2 (30mins)	13.3	253 (30mins)
CONTROL	5.8	23.7 (15mins)	5.0	7.1 (90mins)	12.2	125 (90mins)

Conclusion: GLP-1 response to a mixed meal does not appear exaggerated in NIPHS.

(1) Service GJ et al. NEJM 2005; 353:249-54
(2) Morinigo R et al. JCEM 2006; 91:1735-1740
(3) le Roux CW et al. Annals of Surgery 2006; 243(1):108-114

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AN AUDIT OF TREATMENT OUTCOMES OF GRAVES' DISEASE AND TOXIC MULTINODULAR GOITRE IN SOUTH AUCKLAND

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Thyrotoxicosis associated with significant morbidity and mortality. Graves' Disease (GD) and Toxic Multinodular Goitre (TMNG) account for most cases of thyrotoxicosis.

Aims of this study are to assess short-term treatment outcomes (minimum 6 months) and to determine the demographic data of the patients with GD and TMNG.

A retrospective case-record review of patients with GD and TMNG treated at the outpatient clinics between 1 June 2004 and 31 May 2005 was performed. Diagnosis of GD and TMNG were based on clinical presentation, thyrotropin receptor antibody positivity and supportive nuclear scintiscan findings. Carbimazole (CBZ) was administered for at least 12-18 months. ^{131}I (dose range 400-1000MBq) was given as a single dose. Remission was defined as clinical and biochemical euthyroid status for at least six months post-treatment.

A total of 90 patients (72 GD patients and 18 TMNG) comprising 18 males and 72 females were studied. Mean age was 50yrs (range 20-94yrs). Median duration of CBZ treatment was 12 months. The ethnic distribution of GD was: Maori,15%; Pacific, 29%; Asian,11%; and others, 44%. The ethnic distribution of TMNG was: Maori, 28%; Pacific, 11%; Asian 0%; and others, 61%. 21 radio iodine treated patients developed permanent hypothyroidism.

Treatment Modality	GD	TMNG	Total
Carbimazole	37 (11)*	3 (2)	40 (13)
Radio Iodine (^{131}I)	32 (1)	13 (0)	45 (1)
Surgery	3 (0)	2 (0)	5 (0)
Total	72	18	90

Table 1: Summary of treatment outcome

* Number of patients with treatment failure given within brackets.

Conclusions: TMNG was the predominant cause of thyrotoxicosis among NZ Maori and Europeans and GD was the predominant cause among Pacific people and Asians. Short term remission rate was 70 % in CBZ treated GD patients. 131I treatment achieved a higher cure rate for GD and TMNG. These results suggest 131I should be the primary mode of treatment for TMNG and GD if there are no contraindications to this therapy. Further studies are needed to confirm this observation.

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