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- **ESA – SRB PROGRAM & LOCAL ORGANISING COMMITTEES**
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  - Adelaide Convention Centre Venue Map Layout
- **PROGRAM**
  - Sunday 23rd August
  - Monday 24th August
  - Tuesday 25th August
  - Wednesday 26th August
- **ESA - SRB POSTER LISTING**
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- **NOTES**
Quadripolar immunofluorescence of 16.5 dpc mouse ovary showed for the first time that the adult stem cell marker LGR5 (yellow), the forkhead transcription factor FOXL2 (green), the nuclear receptor NR2F2, also known as COUP-TFII (red) that the expression of these factors is mutually exclusive and therefore mark different somatic cell populations in the foetal mouse ovary. To distinguish from somatic cells, germ cells were marked with MVH (light blue) and cell nuclei with DAPI (dark blue).

Raphael H Rastetter and Dagmar Wilhelm
Ann McCormack (ESA Chair)
*Department of Endocrinology, St Vincent’s Hospital, Garvan Institute of Medical Research*

Dr McCormack is a staff specialist in the Department of Endocrinology at St Vincent’s Hospital and holds a conjoint position at the Garvan Institute of Medical Research and The University of New South Wales. Her primary research interest is in the field of pituitary tumour biology, in particular aggressive pituitary tumours. She completed her PhD studies at the Kolling Institute of Medical Research examining the role of MGMT (06-methylguanine-DNA methyltransferase) as a biomarker of response to temozolomide (a novel therapy for aggressive pituitary tumours) and exploring its role in pituitary tumorigenesis. She was awarded the 2008 ESA Clinical Endocrinology Award and has been invited to present at the 2009 American Endocrine Society Annual Scientific Meeting at the 2012 International Congress of Endocrinology in Italy and in 2013 at the International Pituitary Congress. During 2010, she has also undertaken a research and clinical fellowship at the Oxford Centre for Diabetes, Endocrinology and Metabolism in the UK, a centre with an international reputation for expertise in pituitary disease and neuroendocrine tumours. She has recently been awarded the 2013 John Shine Translational Research Fellowship at the Garvan Institute.

Rebecca Robker (SRB Co-Chair)
*Robinson Institute; University of Adelaide*

Dr Rebecca Robker is an NHMRC RD Wright Biomedical Research Fellow at The University of Adelaide and The Robinson Institute where she leads the Ovarian Cell Biology Laboratory. She received her PhD from Baylor College of Medicine (Houston TX USA) in 1999 and was recruited to the University of Adelaide in 2003. Her research is focused on identifying cellular pathways in the ovary that ensure a good egg at the right time. In particular her lab is discovering key genes regulated by the progesterone receptor nuclear transcription factor that are essential for ovulation and identifying how obesity causes anovulation and altered embryo growth.

Kaye Stenvers (SRB Co-Chair)
*Hudson Institute of Medical Research, Clayton*

Dr. Stenvers (PhD, UNC-Chapel Hill) is head of the Reproductive Development and Cancer Laboratory at Prince Henry's Institute and an adjunct lecturer with the Department of Anatomy and Developmental Biology, Monash University. She did her postdoctoral training at the Ludwig Institute for Cancer Research (Melbourne) before moving to moving to Prince Henry's Institute, now the Hudson in 2005. Her research currently focuses on defining the roles of TGFβ superfamily members in the development of both the testis and ovary and in the pathogenesis of reproductive cancers. Her major research contributions are to the understanding of the functions of the type III TGFβ receptor, TGFBR3. She was the first to generate and characterise the TGFBR3 null mouse line and is currently establishing new roles for this receptor as a tumour suppressor in human ovarian cancers.

The ESA-SRB POC chairs wish to thank all those assisting the POC chairs in abstract reviewing for the 2015 ASM. We would especially like to thank the committees headed by Chris O’Neill, Brett Nixon and Ann McCormack who selected the award finalists. We also wish to thank the ESA-SRB LOC members Nicolette Hodyl (ESA LOC Chair), Wendy Ingman (SRB LOC Chair), Hannah Brown, Morton Burt, Kathy Gatford, Anjana Radhakutty and Amy Wooldridge.
ESA PROGRAM ORGANISING COMMITTEE

Ann McCormack (Chair)  
St Vincent’s Hospital and Garvan Institute of Medical Research

Timothy Cole  
Monash University

Sue Mei Lau  
Prince of Wales Hospital

Paul Baldock  
Garvan Institute of Medical Research

Mark Cooper  
Concord Hospital

Chris Ormanny  
Garvan Institute of Medical Research

Morton Burt  
Flinders Medical Centre

Kathy Gatford  
Robinson Research Institute

Anjana Radhakutty  
Lyell Mc Ewin Hospital

Rory Clifton-Bligh  
Royal North Shore Hospital and Kolling Institute of Medical Research

Mathis Grossmann  
University of Melbourne, Austin Health

Renea Taylor  
Monash University

Nicolette Hodyl  
Robinson Institute, University of Adelaide

Amy Wooldridge  
Robinson Institute, University of Adelaide

ESA LOCAL ORGANISING COMMITTEE

Nicolette Hodyl (Chair)  
Robinson Institute, University of Adelaide

Kathy Gatford  
Robinson Research Institute

Amy Wooldridge  
Robinson Institute, University of Adelaide

Morton Burt  
Flinders Medical Centre

Anjana Radhakutty  
Lyell Mc Ewin Hospital

SRB PROGRAM ORGANISING COMMITTEE

Rebecca Robker (SRB co-chair)  
Robinson Institute; University of Adelaide

Lisa Akison  
University Of Queensland

Melanie Sutton-McDowall  
University of Adelaide

Kaye Stenvers (SRB co-chair)  
Hudson Institute of Medical Research, Clayton

Peter Mark  
University of Western Australia

Jeremy Thompson  
University of Adelaide

Brett Nixon (Awards)  
The University of Western Australia

Jeremy Smith  
The University of Western Australia

Tu’Uhevaha Kaitu’u-Lino  
University of Melbourne

Linda Wu  
Robinson Research Institute

SRB LOCAL ORGANISING COMMITTEE

Wendy Ingman (Chair)  
University of Adelaide

Hannah Brown  
Robinson Research Institute

‘MAKING BABIES IN THE 21ST CENTURY’ PUBLIC SYMPOSIUM COMMITTEE

Rebecca Robker  
University of Adelaide

Sarah Meachem  
Hudson Institute of Medical Research

Melanie Sutton-McDowell  
SRB Publicity Secretary, University of Adelaide

Hannah Brown  
University of Adelaide

Leigh Nicholson  
University of Sydney

John Schjenken  
University of Adelaide

Jemma Evans  
Hudson Institute of Medical Research
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<td>P. Taft</td>
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<td>K. Ferguson</td>
<td>T. J. Martin</td>
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<td>K. Ferguson</td>
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<td>1976-78</td>
<td>S. Posen</td>
<td>J. P. Coghlan</td>
<td>P. E. Harding</td>
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<td>1978-80</td>
<td>J. P. Coghlan</td>
<td>C. J. Eastman</td>
<td>R. G. Larkins</td>
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<td>1980-82</td>
<td>C. J. Eastman</td>
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<td>D. P. Cameron</td>
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<td>R. C. Baxter</td>
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<td>S. J. Judd</td>
<td>J. R. Stockigt</td>
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<td>J. R. Stockigt</td>
<td>J. A. Eisman</td>
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<td>1996-98</td>
<td>D. J. Topliss</td>
<td>R. J. Rodgers</td>
<td>G. P. Risbridger</td>
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<td>R. J. Rodgers</td>
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<td>K. K. Y. Ho</td>
<td>B. J. Waddell</td>
<td>B. Canny</td>
<td>C. Coulter</td>
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<td>2002-03</td>
<td>B. Canny</td>
<td>J. D. Zajac</td>
<td>R. Cuneo</td>
<td>C. Coulter</td>
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<td>2004-06</td>
<td>J. D. Zajac</td>
<td>L. Bach</td>
<td>M. McLean</td>
<td>V. Clifton</td>
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<td>B. Yeap</td>
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<td>V. Clifton (Feb 10)</td>
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<td>P. Ebeling</td>
<td>T. Cole</td>
<td>W. Inder</td>
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<td>H. Teede</td>
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<td>2013-14</td>
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<td>H. Teede</td>
<td>T. Cole</td>
<td>W. Inder</td>
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SPONSORS OF THE ENDOCRINE SOCIETY OF AUSTRALIA

ESA AWARD SPONSORS

KEITH HARRISON MEMORIAL LECTURERS

The Keith Harrison Memorial Lecture is given each year at the ESA Annual Scientific Meeting in honour of Prof Keith Harrison, one of the founders of ESA and an early President.

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
2007 Gerard Karsenty
2008 Colin Ward
2009 John Cidlowski
2010 Stafford Lightman
2011 Paul Stewart
2012 Lucilla Poston
2013 Matthew During
2014 Sundeep Khosla
2015 Richard Santen

PINCUS TAFT MEMORIAL LECTURES

Pincus was a founder of clinical endocrinology in Melbourne and in Australia. As the obituary states he was the inspiration and role model for many younger colleagues to take up endocrinology. He had a rare ability to accord respect to all his colleagues from interns and JRMOs through to contemporaries while still being able to instruct or guide them according to an uncompromisingly high standard and he was never afraid to openly acknowledge someone else's ideas if he thought they were better than his [although not a common occurrence].

1994 C Ronald Kahn
1995 William Bremner
1996 Steven Lamberts
1997 George Brabant
1998 Simeon Taylor
1999 Christopher K Glass
2001 Domenico Accili
2002 Paul Stewart
2003 Terry Davies
2004 Peter E Clayton
2005 David Dunger

2006 Sadaf Farooqi
2007 Robert J Smith
2008 William F Young Jr
2009 Karen Miller
2010 Karel Pacak
2011 Kathleen Hoeger
2012 Gudmundur Johannsson
2013 Anthony Hollenberg
2014 John Wass
2015 Michael Tuttle
The Novartis Junior Investigator Award is awarded annually to a member who is a postgraduate student or recently graduated post-doctoral fellow, 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 Rena 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  
2007 Marianne Elston  
2008 Sue Lau  
2009 Kenneth Ho  
2010 Lyndal Tacon  
2011 Jun Yang  
2012 Patrick Candy  
2013 Kevin Lee Tao-Kwang  
2014 Marianna Volpert

The ESA Bryan Hudson Clinical Endocrinology Award is awarded annually to recognise the best clinical research presentation at the Annual Scientific Meeting by an active member of the Endocrine Society of Australia early in their career.

2004 Sonia Davison  
2005 Carolyn Allan  
2006 Jui Ho  
2007 Morton Burt  
2008 Ann McCormack  
2009 Paul Lee  
2010 Jeremy Hoang  
2011 Lucia Gagliardi  
2012 Caroline Jung  
2013 Emily Gianatti  
2014 Phillip Wong

This award supports younger members of the society to travel to international meetings, laboratories and/or clinics to further their training and knowledge in Endocrinology.

2003 Emma Ball  
2004 Gordon Howarth, Sophie Chan and Vincenzo Russo  
2005 Stuart Ellem  
2006 Kevin Pfleger and Erosha Premaratne  
2007 Lisa-Marie Atkin, Elspeth Gold and Michael Stark  
2008 Elif Ekinci, Andrew Siebel, Jenny Chow  
2009 Michelle Van Sinderen, Jyotsna Pippal and Ulla Simanainen  
2010 Wee-Ching Kong, Fredrick Steyn, Ann McCormack  
2011 Stacey Jamieson, Kristy Brown, Kevin Knowler  
2012 Christopher Yates, Dana Briggs, Shyuan Ngo, Sarah To  
2014 Malgorzata Brzozowska-European, Kelly Walton-International  
2015 Sally Abell-European, Sybil McAuley-International, Jaesung Peter Choi-International
The Servier Award is made annually to recognise the best scientific paper published in the 12-month period preceding the closing date for abstracts for the Annual Scientific Meeting by an active member of the Endocrine Society of Australia early in their career.

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  
2007 Kirsten McTavish  
2008 Belinda Henry  
2009 Kristy Brown  
2010 Zoe Hyde  
2011 Stefan Bagheri-Fam  
2012 Priya Sumithran  
2013 Jennifer Lo  
2014 Anthony Bird  
2015 Christian Girgis

The ESA Mid-Career Research Award recognises an outstanding mid-career researcher in endocrinology.

2009 Rachel Davey  
2010 Peter Liu  
2011 Mathis Grossmann  
2012 Emma Duncan  
2013 Zane Andrews  
2014 Kevin Pfleger  
2015 Lisa Moran

The ESA Senior Plenary Award recognises an outstanding research career in the field of Endocrinology in Australia.

2011 Ken Ho  
2012 Gail Risbridger  
2013 Geoffrey Tregear  
2014 Iain Clarke  
2015 Evan Simpson

The WE / ESA Australian Women in Endocrinology (AWE) Travel Awards recognize outstanding achievements of women in the field of endocrinology.

2001 Karen Kroger, Elizabeth Nye  
2002 Aylin Hanyaloglu, Kylie Hewitt  
2003 Nicola Solomon, Carolyn Allan  
2004 Renea Jarred, Rachel Hill  
2005 Teresa Hickey, Agnes Kovacic  
2006 Rebecca Robker, Nichola Thompson  
2007 Sue Mei Lau, Ashwini Chand  
2008 Johanna Barclay, Ulla Simanainen, Kathryn Backholer  
2009 Kesha Rana, Stacey Jamieson, Kavitha Iyer, Vita Birzniece  
2010 Sarah To, Liza Phillips  
2011 Jun Yang, Shirin Hussain  
2012 Lili Huang, Kara Britt  
2013 Anju Joham, Helen Barrett  
2014 Kathryn Hackman, Sarah Gastras  
2015 Amanda Rickard, Ying Wan

The ESA Ken Wynne Memorial Postdoctoral Research Award is given to an outstanding postdoctoral researcher in endocrinology.

2013 Lisa Moran  
2014 Kristy Brown
### ESA POSTDOCTORAL AWARD

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<td>Jeremy Smith</td>
<td>2013</td>
<td>Radhika Seimon</td>
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<tr>
<td>2010</td>
<td>Sarah Spencer</td>
<td>2014</td>
<td>Lucia Gagliardi</td>
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<td>2012</td>
<td>Kelly Walton</td>
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### ESA RESEARCH HIGHER DEGREE SCHOLARSHIP

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<td>2012</td>
<td>Tilenka Thynne</td>
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<td>2008</td>
<td>Lucia Gagliardi</td>
<td>2013</td>
<td>Carmela Caputo</td>
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<td>2009</td>
<td>Pui Shi (Tammy) Pang</td>
<td>2014</td>
<td>Dily Leung</td>
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### ESA HONORARY LIFE MEMBERS

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<td>Prof Terry J. Robinson</td>
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<td>Dr Alan W. Blackshaw</td>
<td>Dr Philip Harding</td>
<td>Prof Rodney Shearman</td>
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<td>Dr Hal D. Breidahl</td>
<td>Prof Basil Hetzel</td>
<td>Dr Evan Simpson</td>
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<td>Prof James B Brown</td>
<td>Dr Brian Hirschfeld</td>
<td>Prof Alfred W Steinbeck</td>
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<td>Prof Henry G Burger</td>
<td>Prof Ken Ho</td>
<td>Prof Jim Stockigt</td>
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<td>Dr Robin A. Burston</td>
<td>Bryan Hudson</td>
<td>Prof Roderick Strang</td>
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<td>Dr Ivan G Jarrett</td>
<td>Prof Pincus Taft</td>
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<td>Prof Leslie Lazarus</td>
<td>Prof Prof Victor Trikojus</td>
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<td>Prof Ian McDonald</td>
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<td>Dr Ian B Hales</td>
<td>Prof Gail Risbridger</td>
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Adelaide Research Assay Facility (ARAF)

Providing specialised high-throughput assays

About ARAF
ARAF provides specialised, high-throughput and high-sensitivity assays of physiologically important analytes for academic researchers and commercial customers Australia-wide.

The facility provides a one-stop-shop for researchers who require these analyses but who do not have expertise, reagents, equipment or appropriately trained and experienced personnel to undertake them.

ARAF services
- Assay of analytes by conventional RIA using commercially available kits or reagents (double antibody and coated tube) in biological fluids or cell/tissue extracts
- Assay of analytes by conventional ELISA using commercially available kits
- Multiplexed ELISAs using xMAP technology
- Assay of challenging analytes using unique validated in-house assays, e.g. melatonin
- Assay of plasma/serum glucose, NEFA, triglycerides, cholesterol, etc. using a Cobas Integra 400 Plus chemistry analyser
- A consultation service to assist in choosing the appropriate reagents/kits or in developing new assays for analytes in non-human species

Contact us
For more information visit our website or contact Mark to discuss services and to seek a quote:

Mark Salkeld / Facility Manager
+61 8 8313 4090
mark.salkeld@adelaide.edu.au
adelaide.edu.au/robinson-research-institute/research/facilities/ARAF
### SRB OFFICE BEARERS 2015

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<td>2013 - 14</td>
<td>Susan Fisher</td>
</tr>
<tr>
<td>2012 - 13</td>
<td>Keith Jones</td>
<td>2014 - 15</td>
<td>Gerald Schatten</td>
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### SRB - JSA / David Healy New Investigator Award

<table>
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<tr>
<th>Years</th>
<th>Name</th>
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<tbody>
<tr>
<td>1982 - 83</td>
<td>RJ Rodgers &amp; CB Gow</td>
<td>1999 - 00</td>
<td>E Whiteside</td>
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<td>1983 - 84</td>
<td>SP Flaherty</td>
<td>2000 - 01</td>
<td>CE Gargett</td>
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<td>1984 - 85</td>
<td>C O’Neill</td>
<td>2001 - 02</td>
<td>WV Ingman</td>
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<td>1985 - 86</td>
<td>BJ Waddell</td>
<td>2002 - 03</td>
<td>C Smith</td>
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<td>1986 - 87</td>
<td>LJ Wilton</td>
<td>2003 - 04</td>
<td>AN Sferruzzi-Penri</td>
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<td>1987 - 88</td>
<td>A Stojanoff</td>
<td>2004 - 05</td>
<td>K Webster</td>
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<td>1988 - 89</td>
<td>MB Harvey</td>
<td>2005 - 06</td>
<td>T Hickey</td>
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<td>1989 - 90</td>
<td>AH Toney</td>
<td>2006 - 07</td>
<td>K Walters</td>
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<td>1990 - 91</td>
<td>H Massa</td>
<td>2007 - 08</td>
<td>C Hogarth</td>
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<td>1992 - 93</td>
<td>SW Walkden-Brown</td>
<td>2009 - 10</td>
<td>AS Care</td>
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<td>1993 - 94</td>
<td>CM Markey</td>
<td>2010 - 11</td>
<td>P Nichols</td>
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<td>MJ Hötzel, S McDougall</td>
<td>2011 - 12</td>
<td>A Reid</td>
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<td>1995 - 96</td>
<td>I van Wezel</td>
<td>2012 - 13</td>
<td>Y R Gao</td>
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<tr>
<td>1996 - 97</td>
<td>S Robinson</td>
<td>2013 - 14</td>
<td>A Winship</td>
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### SRB - Robinson Research Institute Award for Research Excellence

<table>
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<tr>
<td>2006 - 07</td>
<td>M O’Bryan</td>
<td>2011 - 12</td>
<td>C Gargett</td>
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<tr>
<td>2007 - 08</td>
<td>E McLaughlin</td>
<td>2012 - 13</td>
<td>B Nixon</td>
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<td>2008 - 09</td>
<td>SA Robertson</td>
<td>2013 - 14</td>
<td>J St. John</td>
</tr>
<tr>
<td>2009 - 10</td>
<td>RB Gilchrist</td>
<td>2014 - 15</td>
<td>R Robker</td>
</tr>
<tr>
<td>2010 - 11</td>
<td>E Dimitriades</td>
<td>2015 - 16</td>
<td>M Baker</td>
</tr>
</tbody>
</table>
ESA-SRB AWARDS 2015

ESA SERVIER AWARD WINNER

Christian Girgis
Dr Christian Girgis is an NHMRC post-doctoral research fellow at the Centre of Diabetes, Obesity and Endocrinology at Westmead Millennium Institute, Sydney. His PhD at the Garvan Institute examined roles of vitamin D in skeletal muscle and helped to clarify longstanding controversies in this field. He is a visiting scholar at the Salk Institute of Biological Studies, La Jolla USA and a staff endocrinologist at Westmead Hospital, Sydney.

ESA MID-CAREER AWARD WINNER

Lisa Moran
Dr Lisa Moran is a National Heart Foundation Post-Doctoral Research Fellow with The Robinson Research Institute, The University of Adelaide and Monash Centre for Health Research and Implementation, Monash University and an Accredited Practising Dietitian. Her research focuses on optimising management of obesity and related comorbidities in women’s health, specifically the assessment and evidence-based management of the reproductive, metabolic and psychological health of women of reproductive age: (1) Pre-pregnancy, (2) during pregnancy (3) with lifestyle-related diseases such as polycystic ovary syndrome. Her past research has included the effects of weight loss on fertility, the effects of lifestyle interventions during pregnancy and the effect of weight loss and modifying diet composition on the features of PCOS.

ESA SENIOR PLENARY AWARD WINNER

For the last 30 years his group has researched aromatase, the enzyme responsible for oestrogen biosynthesis. They characterised the human gene and found that it was regulated in a tissue-specific fashion by the use of tissue-specific promoters, in particular in the ovary, placenta and adipose tissue. This enabled an understanding of many aspects of the role of oestrogens in health and disease, for example, their role in postmenopausal breast cancer and male bone metabolism. Simpson has some 500 peer-reviewed articles, almost 30,000 citations and an H-index of 95. He has trained some 70 post-graduate students and fellows, many of whom have gone on to establish their own successful careers.
SRB - THE ROBINSON RESEARCH INSTITUTE AWARD FOR EXCELLENCE IN REPRODUCTIVE BIOLOGY RESEARCH

Mark Baker
Dr. Baker did his PhD in cancer research at Monash University, then moved fields into reproduction under the leadership of L/Prof. John Aitken. He established the University of Newcastle mass spectrometry facility and went about characterising the proteome of spermatozoa. Marks interest lies with understanding male infertility and as such, uses quantitative mass spectrometry to compare fertile and infertile sperm cells. He has established a suite of proteins that not only point to the aetiology of this condition, but can also be used as a definitive diagnosis.

NEWCASTLE REPRODUCTION EMERGING RESEARCH LEADER AWARD FINALISTS

Kirsty Pringle
Dr Kirsty Pringle obtained her PhD in 2008 from the University of Adelaide where her studies focussed on the molecular regulators of placentation. She then began her postdoc at HMRI and the University of Newcastle and has since established a research program focusing on various tissue renin angiotensin systems (RAS) in pregnancy and female reproductive health. She is particularly interested in 1) the intrarenal RAS as an early marker of renal dysfunction in Indigenous pregnant women (Kirsty is CIA on an NHMRC project grant to investigate this), and 2) the role of the intrauterine RAS in maintaining the fetal membranes during pregnancy and protecting from preterm labour.

Tanya Soboleva
Dr Tatiana (Tanya) Soboleva received her BSc (Hons) at Moscow State University, Russia. She was then awarded an International Postgraduate Research Scholarship by the Australian Government. She undertook her PhD study at the John Curtin School of Medical Research at the ANU, investigating nucleocytoplasmic transport of de-ubiquitilating enzymes and developing a novel system for expression and purification of recombinant proteins. In 2006 she joined Prof. David Tremethick’s laboratory at the ANU and discovered a novel histone variant, which is involved in the activation of genes expressed during specific stages of spermatogenesis. Currently, she is establishing herself as an independent researcher with the aims to gain deeper understanding of epigenetic regulation of spermatogenesis and the role of testis-specific epigenetic factors overexpression in cancer.

Pradeep Tanwar
Dr Pradeep Tanwar is an ARC Future Fellow, a Cancer Institute NSW Career Development Fellow and a tenured senior lecturer in the University of Newcastle. He is the group leader of the Gynaecology Oncology program at the University of Newcastle and Hunter Cancer Research Alliance. His research focuses on defining molecular footsteps involved in the pathogenesis of the reproductive tract cancers. In 2015, Dr Tanwar was awarded with the best abstract presented by a clinician award at the annual scientific meeting of the Australia New Zealand Gynaecological Oncology Group (ANZGOG). In the same year, his research work on ovarian cancer was profiled by the Cancer Institute NSW to celebrate ovarian cancer research month. The significance of his research work is further recognised by the peer-reviewed grant funding support (~3million/3yrs) including grants from the NHMRC, the ARC and the Cancer Institute NSW.

Kristy Walters
Dr Kirsty Walters was awarded her PhD in 2005 from the University of Edinburgh, Scotland for her work on the role of the insulin-like growth factor (IGF) system in ovarian follicular development. Subsequently, she was recruited to the ANZAC Research Institute, Sydney, Australia where her research focuses on the role of androgens in female reproduction and polycystic ovary syndrome (PCOS). Her research has been recognized by awards including the Society for Reproductive Biology Young Investigator Award (2006), Australian Menopause Society Young Investigator Award (2009), and the University of Sydney Dean's Early Career Researcher Award (2011), and funding as Principal Investigator in two grants from the National Health and Medical Research Council (NHMRC) and an Australian Research Council (ARC) Discovery Early Career Researcher Award (2012).
Somatuline® autogel®
Lanreotide

A convenient way to treat patients with acromegaly¹

- READY TO INJECT IN A PRE-FILLED SYRINGE¹

- AUTOMATIC SAFETY SYSTEM¹

PBS Information: Authority required (STREAMLINED for Public Hospitals only). This product is a Highly Specialised Drug listed on the PBS as a Section 100 item. Refer to PBS schedule for full authority information.

Before prescribing please refer to full Product Information (http://secure.healthlinks.net.au/content/ipsen/pi.cfm?product=ipsatgj)

Somatuline® Autogel®: Lanreotide as acetate in a pre-filled syringe (60, 90 and 120 mg) fitted with an automatic safety system. **Indications:** 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 or other excipients. **Precautions:** May experience hypoglycaemia or hyperglycaemia (monitor blood glucose levels); slight decrease in thyroid function; may reduce gall bladder motility (recommend gall bladder cholography); exclude presence of obstructive intestinal tumour; monitor kidney and liver function; may reduce heart rate in patients without an underlying cardiac problem (monitor heart rate; caution with treatment initiation in patients with bradycardia). Not recommended for use in children. See full PI for further information. **Interactions with Other Medicines:** Reduced absorption of cyclosporin A, decreased bioavailability of cyclosporine, increased availability of bromocriptine, additive bradycardia effects with beta-blockers, decreased clearance of quinidine, terfenadine. **Effect on driving/using machinery:** If affected by dizziness do not drive or use machinery. **Adverse Events:** Common: Common: diarrhea, headache, abdominal pain, cholelithiasis, flatulence, dizziness, headache, sinus bradycardia, alopecia, hypotrichosis, hypoglycaemia, nausea, vomiting, dyspepsia, flatulence, abdominal distension, abdominal discomfort, constipation, biliary distension, injection site reaction (pain, mass, induration, nodules, pruritus), laboratory investigation changes. 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 (healthcare professional or carer); or the upper, outer thigh (self-administration). **Decision for injection by patient or carer to be made by a healthcare professional. Patients must be counselled on Somatuline Autogel and patients/carers must be motivated, competent and trained to inject. **Storage:** 2°C–8°C. **Date of most recent amendment:** 23 July 2013

Reference 1. Somatuline Autogel Product Information. Date of most recent amendment: 23 July 2013. For further information, contact Ipsen Pty Ltd. T (03) 8544 8100 F (03) 9562 5152 E info@ipsen.com.au Level 2, Building 4, Brandon Office Park, 540 Springvale Road, Glen Waverley, VIC 3150 Australia Ipsen Pty Ltd, ABN 47 095 036 909 Date of preparation July 2014 Wellmark IPS24323

Ipsen
Innovation for patient care
Prof Richard Santen  
*University of Virginia, USA*

Dr. Richard Santen is a Professor of Medicine in the Division of Endocrinology at the University of Virginia and has an active clinical practice. His research interests have focused on the development of aromatase inhibitors for treatment of breast cancer, mechanisms relating estrogens to breast cancer, the biology and natural history of endocrine-dependent breast cancer, and the effects of vaginal estrogens on circulating hormone levels. He has published over 400 manuscripts and chapters, predominantly related to the role of estrogen in breast cancer development and treatment. He has been funded consecutively by the National Institutes of Health for over three decades. For his work in the development of aromatase inhibitors, he received the Susan Komen Foundation Brinker International Award for breast cancer clinical research. Other awards include the Clinical Chemistry Distinguished Science Award, the Robert H. Williams Distinguished Leadership Award of the Endocrine Society, and the William L. McGuire Memorial Lectureship Award for breast cancer. Elected professional memberships include the American Society for Clinical Investigation and the Association of American Physicians. He is a long standing member of the American Society of Oncology (ASCO). He recently became President of the Endocrine Society, an organization with 17500 members.

Prof Michael Tuttle  
*Memorial Sloan Kettering Cancer Center, USA*

Prof R Michael Tuttle, an Endocrinologist and Professor of Medicine at Memorial Sloan Kettering Cancer Center in New York, designed and validated the first real time risk assessment model for thyroid cancer management in which individual risk estimates are modified over time as a function of response to initial therapy. In addition, his clinical studies have helped to define the role of recombinant human TSH as an adjuvant for radioactive iodine therapy in both low risk and high risk patients and in the setting of radioactive iodine avid distant metastases. His research continues to center on important management aspects of thyroid cancer including efforts to better define risk of recurrence, risk of death, and the potential role for observation in low risk thyroid cancer. His clinical practice is entirely devoted to patients with thyroid cancer with a particular emphasis on cases with aggressive disease and complicated management issues.

Prof Steven Kahn  
*University of Washington, USA*

Steven E. Kahn, MB, ChB is a Professor of Medicine in the Division of Metabolism, Endocrinology and Nutrition at the VA Puget Sound Health Care System and University of Washington in Seattle, Washington. He holds the Leonard L. Wright and Marjorie C. Wright Chair in Diabetes and directs the Diabetes Research Center at the University of Washington. Dr. Kahn’s research interests include the role of β-cell in the pathogenesis and treatment of prediabetes and type 2 diabetes. He has performed physiological studies characterizing β-cell function in individuals with diabetes and those at increased risk and is an active participant in a number of large multicenter clinical trials in which interventions are being tested to prevent and treat type 2 diabetes. Aside from his clinical studies, he has an extensive basic research program examining the role of islet amyloid in the loss of β-cells in type 2 diabetes. He has received numerous awards for his research including the Novartis Young Investigator in Diabetes Award, American Diabetes Association Distinguished Clinical Scientist Award, The Endocrine Society Clinical Investigator Award, United States Department of Veterans Affairs John B. Barnwell Award, and European Association for the Study of Diabetes Albert Renold Award.
**ESA PLENARY LECTURER**

Dr William Rainey  
*University of Michigan, USA*

Dr. William (Bill) Rainey is the Jerome Conn Professor in the Departments of Molecular and Integrative Physiology and Internal Medicine at the University of Michigan where he also serves as the Director of Endocrine Neoplasia Basic Research. Dr. Rainey is an internationally recognized endocrine researcher specializing in adrenal zonation, steroid production and tumor development. Rainey’s recent application of genomic and metabolomic methods to adrenal research has helped define the role of gene mutations in causing adrenal neoplasias and facilitated the discovery of novel steroid biomarkers for adrenal diseases. His adrenal research program has been continuously funded by the National Institutes of Health and American Heart Association for the past 30 years. During that time, Rainey has authored or co-authored over 200 scientific publications and has served as the American Editor for the journal Molecular and Cellular Endocrinology for 15 years.

**SRB FOUNDER’S LECTURER**

Prof Blanche Capel  
*Duke University Medical Center, USA*

Blanche Capel, PhD, is a James B. Duke Professor of Cell Biology at Duke University Medical Center, where she began her research laboratory in 1993. Her graduate training was in mouse genetics and stem cell biology with Beatrice Mintz at Fox Chase Cancer Center and the University of Pennsylvania, followed by postdoctoral research in the Lovell-Badge laboratory at the National Institute for Medical Research, Mill Hill, London, leading to the discovery of Sry, the male sex determining gene in mammals. Work from the Capel laboratory is prominent in the field of primary sex determination and the cell fate and patterning decisions that underlie the development of the early bipotential mammalian gonad into either testis or ovary. Dr. Capel’s work on signaling pathways in the gonad led to the widely accepted current model that sex determination results from antagonism between the male and female transcriptional and cell signaling networks. She pioneered organ culture techniques for studying the organogenesis of the testis and ovary, and was among the first to develop live imaging to explore the critical role of the vasculature in the morphogenesis of the gonad. Recently, she has used systems-biology and mouse genetics approaches to characterize the global transcriptional network underlying gonad fate, and has investigated the conservation of these mechanisms in the red-eared slider turtle, in which sex determination is thermally regulated. Other work in her laboratory aims to understand how the intracellular program in germ cells, in combination with regulation from their niche, leads to the transition of germ cells from a pluripotent state into pro-spermatogonia. This work involves Dnd1Ter, a mouse mutant in which germ cells do not successfully navigate this transition, but instead are transformed to germ cell tumors. Capel is an editor of Developmental Biology, and serves on the editorial boards of Developmental Dynamics and Sexual Development. She was a founding member of the DEV1 study section at NIH, and has served on numerous other NIH and NSF panels as well. She has also served on the boards of the Society for Developmental Biology, the Society for the Study of Reproduction and on the Board of Scientific Advisors at the Jackson Laboratory. She has organized the International Symposium on Vertebrate Sex Determination since 2006, and has been an organizer of the Cold Spring Harbor, Molecular Embryology of the Mouse course, as well as the biannual Cold Spring Harbor Germ Cell Meeting. She is a member of the American Association for the Advancement of Science. She serves as the representative to FASEB from the Society for Developmental Biology.

**SRB PRESIDENT’S LECTURER**

Prof. Moira O’Bryan  
*Monash University, Melbourne*

Moira graduated from The University of Melbourne in 1994, after which she was awarded an Andrew Mellon Foundation Fellowship to work at The Population Council, New York. She returned to Australian, and the Monash Institute of Reproduction and Development, in 1996 as a National Health and Medical Research Council (NHMRC) Peter Doherty Fellow. She is currently a Professor, NHMRC Principal Research Fellow and the Deputy Head of the Department of Anatomy and Developmental Biology at Monash University. The focus of her lab encompasses: sperm and cilia development, genetic causes of human infertility and the implications for ‘reproductive’ proteins on health broadly.
<table>
<thead>
<tr>
<th>ESA-SRB SYMPOSIUM SPEAKERS</th>
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<tbody>
<tr>
<td>Melanie Bagg</td>
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<tr>
<td>Queensland University of Technology, Queensland</td>
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<td>Simon Barry</td>
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<tr>
<td>University of Adelaide, Adelaide</td>
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<td>Kenneth Beagley</td>
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<td>Kiri Beilby</td>
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<td>Origio, Denmark</td>
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<td>Vita Birzniece</td>
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<td>University of Western Sydney, Sydney</td>
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<td>Andrew Brooks</td>
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<td>University of Queensland, Queensland</td>
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<tr>
<td>Kristy Brown</td>
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<td>Hudson Institute of Medical Research, Clayton</td>
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<td>Mark Brown</td>
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<td>South East Sydney and Illawarra Area Health, Sydney</td>
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<td>Morton Burt</td>
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<td>Flinders University, Adelaide</td>
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<td>Nuala Byrne</td>
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<td>Bond University, Queensland</td>
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<td>David Callen</td>
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<td>Pauline Campos</td>
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<td>University of Otago, NZ</td>
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<td>Shuan Chen</td>
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<td>Rory Clifton-Bligh</td>
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<td>Angela Crean</td>
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<td>Robin Daly</td>
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<td>Lucia Gagliardi</td>
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<td>Royal Adelaide Hospital, Adelaide</td>
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<td>Leonie Heilbronn</td>
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<td>Sabrina Heng</td>
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<td>Ken Ho</td>
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<td>Mark Hutchinson</td>
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<tr>
<td>Karla Hutt</td>
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<tr>
<td>Monash University, Melbourne</td>
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<tr>
<td>Karin Jandeleit-Dahm</td>
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<tr>
<td>Baker IDI Heart and Diabetes Institute, Melbourne</td>
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</table>
Christine Jasoni
University of Otago, NZ

Jeff Keelan
University of Western Australia, Crawley

Ken Korach
National Institute of Environmental Health Sciences

Martha Lappas
University of Melbourne, Melbourne

Peter Mark
University of Western Australia, Crawley

Glenn McConell
Victoria University, Footscray

Karen Moritz
University of Queensland, Queensland

Shyuan Ngo
University of Queensland, Queensland

Guiying Nie
Hudson Institute of Medical Research, Clayton

Nick Pocock
University of New South Wales, Sydney

Malcolm Purdey
University of Adelaide, Adelaide

Roger Reddel
Children’s Medical Research Institute, Westmead

Mike Rogers
Garvan Institute of Medical Research, Sydney

Ryan Rose
Fertility SA, Adelaide

Kerry-Anne Rye
UNSW Medicine, Sydney

Richard Saffery
Murdoch Childrens Research Institute, Parkville

Darren Saunders
Garvan Institute of Medical Research, Sydney

Erik Schartner
University of Adelaide, Adelaide

John Schjenken
University of Adelaide, Adelaide

Lisa Schwanz
University of New South Wales, Sydney

Evan Simpson
Hudson Institute of Medical Research, Clayton

Roger Smith
University of Newcastle, Newcastle

Aneta Stefanidis
Monash University, Melbourne

Derik Steyn
University of Queensland, Queensland

Catherine Suter
Victor Chang Cardiac Research Institute, Darlinghurst

Helena Teede
Monash University, Clayton

David Thomas
Garvan Institute of Medical Research, Sydney

Stephen Tong
University of Melbourne, Melbourne

Euan Wallace
Monash University, Clayton

Bu Yeap
University of Western Australia, Perth

Jeffrey Zajac
University of Melbourne, Melbourne

SRB ‘MAKING BABIES IN THE 21ST CENTURY’ SYMPOSIUM SPEAKERS

Michael Davies
Robinson Research Institute, Adelaide

Claire Roberts
University of Adelaide, Adelaide

Robert Norman
Robinson Research Institute, Adelaide

Sarah Robertson
University of Adelaide, Adelaide

Alice Rumbold
University of Adelaide, Adelaide
JANUVIA is the only oral agent for T2DM with CV Safety demonstrated in a 3-year RCT involving 14,000 patients*1,4

*When added to usual care


Life-threatening lactic acidosis can occur due to accumulation of metformin. Risk factors include renal impairment, old age and the use of high doses of metformin above 2000 mg per day.
**INFORMATION FOR DELEGATES & PRESENTERS**

**Venue**
Adelaide Convention Centre
1, 15 Leigh Street, Adelaide SA 5000
Phone: (08) 8212 4099

**Venue Layout**
The registration desk is located in Foyer F on the ground level, next to the main entrance. All catered breaks are taken within Hall H directly behind the registration desk. The plenary lectures will run within Halls L & M on the ground level. You can access the plenary lectures through the exhibition rooms. The concurrent sessions will run on all three levels of the Convention Centre.

**Organiser’s Office and Registration Desk**
The registration desk will be open on Sunday 23rd August from 12:00 PM to 5:30 PM and on Monday 24th, Tuesday 25th & Wednesday 26th August from 7:00 AM - 5:30 PM.

**The Speaker Preparation Room**
Presentations are to be loaded direct to the PC in the speaker preparation room (City Suite 1) on the Upper Level at least a full session in advance of your session. You should bring your talk on a USB, saved in a format for display on a pc within the room. A technician will be on hand to assist with any transfer / loading issues and to help you check your presentation. There are both PCs and Macintosh computers in the speaker preparation room but please note there are no Macintosh computers in the presentation rooms.

**Registration**
The Full Delegate Registration fee includes:
- all delegate materials (name tag, satchel, conference booklet)
- lunches (Monday, Tuesday and Wednesday)
- morning teas (Monday, Tuesday and Wednesday)
- afternoon teas (Sunday, Monday and Tuesday)
- the Welcome Function

The Day Registration fee includes:
- all delegate materials (name tag, satchel, conference booklet)
- lunch for the specified day
- morning tea for the specified day
- afternoon tea for the specified day

**Name Tags**
Delegates are required to wear their name tags to all scientific and catered sessions. Uniformed security is in attendance on the doors of the exhibition area and name tags are required to gain access. Delegates should note that within their name tag pouch will be the specific function tickets they have ordered.

**Poster Viewing**
Delegates with posters can find the correct position for their poster by locating the appropriate abstract number on the display panels. The panels are set up in the Exhibition (Hall H) located behind the registration desk. Use the program reference (or ESA-SRB smart phone APP) to identify your abstract number and poster position. Posters can be mounted on Monday morning and must be removed by afternoon tea on Tuesday. During formal poster discussions (on Monday afternoon), the presenters should attend their poster to answer questions and meet colleagues with similar research interests. The posters are grouped in categories and refreshments will be served.

**Internet Café**
There will be an internet café provided for delegates for the duration of the meeting. The café is located on within the Exhibition (Hall H) adjacent to the SRB booth. There is also complimentary wireless internet throughout the Adelaide Convention Centre.

**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, ESA-SRB smart phone APP or the pocket timetable.

**Message Board** - will be available at the registration desk.
Social Functions

- The **Welcome Function** will be held in Foyer F of the Ground Level at the Adelaide Convention Centre on Sunday evening from 5:30pm. Light refreshments and drinks will be served and the function is complimentary for all registration types. Additional tickets for partners can be purchased from the registration desk.

- The ‘Party like it’s the End of the World!’ **Monday Social** will be held at The WorldsEnd Hotel on Monday evening from 7.30pm. Join us for a fun night of dinner, drinks and dancing at the landmark Adelaide pub, The Worlds End. This pub is conveniently located in the heart of the West End on Hindley St, just a couple of blocks from the Convention Centre. Party on with all your ESA and SRB buddies with a BBQ dinner, a few beers and some live music. This is a social function for all delegates attending the conference. It is for students and scholars alike and is a fantastic opportunity for students to pick the brains of their peers and professors. Dinner & drinks included. **This is a ticketed function** ($35 for students, $55 for others) and tickets must be purchased in advance from the registration desk.

- The **Conference Dinner** will be held on Tuesday evening in the Panorama Ballroom on the Upper Level at the Adelaide Convention Centre. Pre-dinner drinks will be served from 7:00pm for a 7:30pm start. Dress is neat casual. **This is a ticketed function** ($110 per ticket) and they must be purchased in advance from the registration desk.

*Smoking* - is not permitted in the venue.

*Mobile Phones* - Please ensure they are turned off or to silent during any session you attend.

*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.

*Exhibition Scanners*

Some exhibitors at this year’s meeting will have scanners that can be used on the barcode on your name badge to collect your contact information. In essence this is like providing them with an electronic business card with exactly the same information as would be contained on a standard business card – ie. name, phone, email, organisation, position. No information beyond this is collected by the scanner. If you agree to have your badge scanned you are consenting to sharing your contact information with the exhibitors. The exhibitors may use your details to contact delegates but are not permitted to share their information with 3rd parties without the consent of the participant.

*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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**SMART PHONE APP**

The App is displayed in a simple and easy to read format on your phone, iPad, or even your computer. To get the ‘App’, please open the below link in your internet browser on your phone, iPad or laptop.

http://esa-srb-2015.m.asnevents.com.au

The app is web based, no download is required.

The Smartphone/Mobile Device ‘App’ will allow you to:

- View the full conference program
- View all abstracts for the conference
- Save your favourite sessions and plan your day
- Take notes which will then be saved and downloaded from your registration profile.
- Answer poll questions from presenters

To use most of these functions, you will be prompted to “log in” each day. Simply enter the same email & password which you used to register.
PBS information: NovoRapid® is listed on the PBS as a drug for the treatment of diabetes mellitus. Levemir® is listed as a restricted benefit for type 1 diabetes.

Levemir® is indicated for once- and twice-daily use in type 1 and type 2 diabetes.

Before prescribing, please review Product Information available from Novo Nordisk.

For the most up-to-date Product Information, call 1800 668 626.
Sunday 23rd August, 2015

SRB Council Meeting
11:30am - 1:00pm Riverbank Boardroom

Registration Open
12:00pm - 5:30pm Foyer H

SRB Symposium: Novel Mechanisms of Transgenerational Inheritance
1:45pm - 3:15pm Riverbank 2 & 3
Chair: Patrick Western

1:45 PM **Damian Dowling**
Evolution within the mitochondria – adaptation, conflict, and the health implications *abs# 1*

2:15 PM **Angela Crean**
Males deliver more than DNA *abs# 2*

2:45 PM **Richard Saffery**
Establishing epigenetic change as the mediator of fetal programming in humans: are we there yet? *abs# 3*

Public Symposium: Making babies in the 21st Century
3:30pm - 5:30pm Riverbank 2 & 3

**Prof Claire Roberts**
Inequality begins before birth

**Prof Michael Davies** Intergenerational transmission of health inequalities

**Dr Alice Rumbold**
Maternal and child health in our Aboriginal and remote communities

**Prof Robert Norman**
Africa to Adelaide- Poverty, prosperity and pregnancy

**Prof Sarah Robertson**
From one generation to the next - parenting begins before conception

Welcome Function
5:30pm - 7:00pm Foyer F
Monday 24th August, 2015

Registration Open
7:00am - 9:00am

Joint Welcome
8:30am - 8:45am
Chairs: Eileen McLaughlin & Helena Teede

ESA Harrison Plenary Lecture
8:45am - 9:45am
Chair: Helena Teede

8:45 AM Richard Santen
Estrogen paradox: how can estrogen both cause and prevent breast cancer? abs# 4

SRB Orals - Non-coding RNAs in reproduction
8:45am - 10:00am
Chairs: Darryl Russell & Andrew Pask

8:45 AM Marilyn B Renfree
Alternative splicing of HOX genes and their adjacent long noncoding RNAs may regulate sexually dimorphic phallus development abs# 5

9:00 AM Hon-yeung Chan
Paternal miR146a influences the female post-coital inflammatory response to seminal fluid abs# 6

9:15 AM Bihong Zhang
Seminal Fluid Regulates miR155, which Impacts on Treg Cells and Alters Pregnancy Outcomes abs# 7

9:30 AM Shoichi Wakitani
Potential effect of activin on establishment of piRNA machinery in human germ cells abs# 8

9:45 AM Tod Fullston
A paternal high fat diet alters founder sperm microRNA profile and implicates it as part of a candidate epigenetic mechanism underlying paternal programing. abs# 9

SRB Orals - Spermatogenesis
8:45am - 10:00am
Chairs: Tanya Soboleva & Mark Baker

8:45 AM Duangporn Jamsai
RBM5 is required for spermatogonia differentiation abs# 10

9:00 AM Eileen A McLaughlin
Dynamin 2: an essential regulator of male germ cell development abs# 11
9:15 AM  **Diana Micati**  
Delineating the role of Snail transcription factors in the testis  *abs# 12*

9:30 AM  **Hidenobu Okuda**  
A novel transcription factor NKAPL is a germ cell-specific suppressor of the Notch signaling pathway and is indispensable for spermatogenesis.  *abs# 13*

9:45 AM  **Brett Nixon**  
Assessment of capacitation-like changes in the spermatozoa of the Australian saltwater crocodile  *abs# 14*

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**SRB Orals - Fertilisation & early embryogenesis**

8:45am - 10:00am  
**Riverbank 5**

Chairs: Mark Green & Mark Nottle

8:45 AM  **Deirdre L Zander-Fox**  
Exposure to ammonium during pre-implantation embryo development significantly alters offspring phenotype.  *abs# 15*

9:00 AM  **Yonggang Lv**  
Phthalate ester-induced cytoskeletal disruption in early embryogenesis of an Australian native marine invertebrate *Galeolaria gemineoa* (Polychaeta: Serpulidae)  *abs# 16*

9:15 AM  **Chris O'Neill**  
Autocrine ligands activate a canonical immediate early gene and late gene response at the time of embryonic genomic activation in the mouse 2-cell embryo  *abs# 17*

9:30 AM  **Geoffry N De Iuliis**  
Oxidative Stress-Induced Protein Modifications in Spermatozoa and Consequences for Sperm-Oocyte Recognition  *abs# 18*

9:45 AM  **Helana Shehadeh**  
Dietary micronutrient supplementation to a high fat diet reduces sperm oxidative stress and improves fertilisation rates in a mouse model  *abs# 19*

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**ESA Servier Award Lecture**

9:45am - 10:00am  
**Halls L & M**

Chair: Helena Teede  
Session sponsored by  

9:45 AM  **Christian Girgis**  
The vitamin D receptor (VDR) is expressed in skeletal muscle of male mice and modulates 25-hydroxyvitamin D (25OHD) uptake in myofibers  *abs# 20*

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**Morning Tea**

10:00am - 10:30am  
**Hall H**
ESA - Novartis Junior Scientist
10:30am - 11:45am
Halls L & M
Chairs: Helena Teede & Maria Volpert
Session sponsored by

10:30 AM 
CheukMan Cherie Au
Des-acyl ghrelin inhibits oestrogen-stimulated breast tumour growth in vitro and in vivo abs# 21

10:45 AM 
Dilys TH Leung
Combined PPARγ and XIAP treatment sensitises granulosa cell tumours to PPARγ-mediated apoptosis abs# 22

11:00 AM 
Bennet Seow
Comparative Effects of Endogenous and Synthetic Glucocorticoid Steroids during Mammalian Lung Development abs# 23

11:15 AM 
Ying Wan
The refinement of Luteinizing Hormone (LH) output during pubertal maturation is associated with the recruitment of follicle in adult female mice abs# 24

11:30 AM 
Dannielle H Upton
Granulosa cell-specific or global Pten mutations in combination with transgenic FSH expression fails to induce ovarian tumors. abs# 25

SRB Orals - Immune cells & Inflammation
10:30am - 12:00pm
Riverbank 2 & 3
Chairs: Mark Hedger & John Schjenken

10:30 AM 
Brandon R Menzies
Major histocompatibility complex class I genes at the fetal-maternal interface of a marsupial (Macropus eugenii) abs# 26

10:45 AM 
Lachlan M Moldenhauer
Toll-like receptor 4 antagonist (+)-naloxone prevents infection-driven fetal loss and preterm labour in mice abs# 27

11:00 AM 
Peck Y Chin
Novel non-competitive Interleukin-1 receptor antagonist prevents LPS-induced preterm birth abs# 28

11:15 AM 
Sivanjah Indumathy
Delineation of myeloid lineage derived immune cells in the adult mouse testis abs# 29

11:30 AM 
James A Deane
Expression of the Stem Cell Marker mTert Identifies Epithelial, Endothelial and Leukocyte populations in the Mouse Endometrium. abs# 30

11:45 AM 
Viv Perry
Sex specific effects of early gestational diet upon the developing immune system abs# 31
SRB - Oozoa Award Finalists
10:30am - 12:00pm
Chairs: Robert Gilchrist & Deirdre L Zander-Fox
Session sponsored by

10:30 AM  Danniele H Upton
High FSH levels alter oocyte in vitro maturation but not oocyte aneuploidy in a transgenic mouse model. abs# 32

10:45 AM  Dave R Listijono
SIRT2 over-expression reverses ageing-induced decline in oocyte quality abs# 33

11:00 AM  Jacinta H Martin
Permeability Glycoprotein Enhances Cellular Drug Exclusion in the Early Embryo; Upholding DNA Integrity abs# 34

11:15 AM  Brendan Houston
The effect of radiofrequency-electromagnetic radiation on the male germ line abs# 35

11:30 AM  Sally Hall
Electrophilic aldehydes increase free radical production and modify surface proteins in horse spermatozoa abs# 36

11:45 AM  Jackson N Reilly
Next generation sequence analysis of miRNA signatures in mouse epididymal epithelial cells and spermatozoa. abs# 37

SRB Plenary Founder's Lecture
12:00pm - 1:00pm
Halls L & M
Chair: Eileen McLaughlin
Session sponsored by Reproduction, Fertility and Development

12:00 PM  Blanche Capel
The Battle of the Sexes: Establishing male or female fate of the bipotential gonad abs# 38

ESA and ENSA Lunch
12:00pm - 1:00pm
Hall H

ESA Clinical Session: What's new in Pituitary?
12:15pm - 1:00pm
Riverbank 4
Chair: Ann McCormack
12:15pm  Ken Ho
What's new in Pituitary?

SRB Lunch
1:00pm - 2:00pm
Hall H
ESA Symposium: Novel uses of bone therapies in the cancer setting
1:00pm - 2:30pm
Halls L & M
Chairs: Peter Ebeling & Christian Girgis
1:00 PM  **Mike Rogers**
Bisphosphonates and cancer: seeing old drugs in a new light  *abs# 39*
1:30 PM  **David Callen**
How does vitamin D influence the risk of breast cancer?  *abs# 40*
2:00 PM  **David Thomas**
RANKL and giant cell tumor of bone: the growing problem of benign tumours.  *abs# 41*

ESA Clinical Orals - Men’s Health and Case Studies
1:00pm - 2:30pm
Riverbank 4
Chair: Carolyn Petersons
1:00 PM  **Jason Tan**
Low endogenous testosterone levels increase the risk of type 2 diabetes in men, independent of established risk factors  *abs# 42*
1:15 PM  **Benjamin Hsu**
Lower circulating testosterone (T) is a consequence rather than a cause of poor health in older men: the Concord Health and Ageing in Men Project (CHAMP)  *abs# 43*
1:30 PM  **Henry Wong**
Sex hormone binding globulin and free testosterone as predictors of mortality in men with type 2 diabetes  *abs# 44*
1:45 PM  **Bu B Yeap**
Proportion of undercarboxylated osteocalcin and serum P1NP predict incidence of myocardial infarction in older men.  *abs# 45*
2:00 PM  **Jasna Aleksova**
Treating Type 1 Diabetes with Glucocorticosteroids: A case report of PD-1 Receptor inhibition induced Type 1 Diabetes  *abs# 46*
2:15 PM  **Jessica L Stranks**
Like mother like son? Variable expression and phenotype of an inactivating dominant ATP-binding cassette sub-family C member 8 (ABCC8) gene mutation within a single family.  *abs# 47*

SRB AGM
1:30pm - 2:30pm
Riverbank 6 & 7

Afternoon Tea
2:30pm - 3:00pm
Hall H
ESA - US Endocrine Society Symposium: Empowering the next generation of clinical and academic endocrinologists - what are the challenges?
3:00pm - 5:00pm
Halls L & M
Chairs: Emma Duncan - TBC & Duncan Topliss

Panellists:
Dr Kirsty Walters , Medicine, Concord Clinical School, ANZAC Research Institute, The University of Sydney
Dr Anju Joham, Postdoctoral Research Fellow, Monash Centre for Health Research and Implementation, School of Public Health and Preventative Medicine, Monash University

3:00 PM  Helena Teede
Empowerment of the next generation of clinicians and researchers in Endocrinology: What are the challenges? The inaugural joint US Endocrine and ESA symposium. abs# 48

3:30 PM  Richard Santen
Title: Empowering the next generation of endocrinologists: United States perspective abs# 49

4:00 PM  Peter Ebeling
4:30 PM  Forum and discussion

ESA - AWE - Neuroendocrinology Australasia Symposium
3:00pm - 5:00pm
Riverbank 2 & 3
Chair: Beverly Muhlhausler

3:00 PM  Christine Jasoni
Understanding how maternal obesity and fetal neuro-immune interactions change the development of the hypothalamic arcuate nucleus in the mouse abs# 50

3:30 PM  Pauline Campos
New technologies to resolve old questions : Using optogenetics to elucidate GnRH/LH pulses abs# 51

4:00 PM  Shyuan Ngo
Neurodegeneration: an endocrine and metabolic perspective abs# 52

4:30 PM  Aneta Stefanidis
TBC abs# 53

SRB - ANZPRA Symposium: Understanding and treating pre-eclampsia
3:00pm - 5:00pm
Riverbank 6 & 7
Chairs: Claire Roberts & Kirsty Pringle

3:00 PM  Euan Wallace
Curing preeclampsia: beyond blood pressure control abs# 54

3:30 PM  Stephen Tong
A systematic screening approach to identify therapeutics for preeclampsia abs# 55
4:00 PM  **Mark Brown**  
Clinical Management of Pre-eclampsia *abs# 56*

4:30 PM  **Guiying Nie**  
Understanding of early-onset preeclampsia through a placenta-specific protease *abs# 57*

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**ESA Basic Symposium: Breast cancer and oestrogen**  
3:00pm - 5:00pm  
Riverbank 4  
Chairs: Peter Fuller & Christopher Ormandy

3:00 PM  **Evan Simpson**  
Metabolism strikes back: metabolic flux regulates cell signalling and proliferation *abs# 58*

3:30 PM  **Shiuan Chen**  
SERPINA1 is a direct Estrogen Receptor target gene and a predictor of survival in breast cancer patients *abs# 59*

4:00 PM  **Kenneth Korach**  
Genetically Modified Mouse Models to Dissect the Physiological Roles of ERα’s Functional Domains *abs# 60*

4:30 PM  **Kristy Brown**  
Dysregulated metabolism in obesity and breast cancer: Clues to novel therapeutics strategies? *abs# 61*

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**SRB Symposium: Nanoscale biosensing in reproductive medicine**  
3:00pm - 5:00pm  
Riverbank 5  
Chairs: Jeremy Thompson & David Gardner

3:00 PM  **Mark Hutchinson**  
New windows into the body *abs# 62*

3:20 PM  **Ewa Goldys**  
Through the looking glass: what can we see in the early embryo when we look carefully enough *abs# 63*

3:40 PM  **Brant Gibson**  
Nanodiamond for BioPhotonic and Hybrid-Photonic applications *abs# 64*

4:00 PM  **Sabrina Heng**  
Microstructured Optical Fibers and Photoswitches: Light-Driven Sensors for Metal Ions in Biology. *abs# 65*

4:20 PM  **Erik Schartner**  
Development of optical fibre probes for biosensing applications *abs# 66*

4:40 PM  **Malcolm S Purdey**  
A Non-invasive Sensor for Hydrogen Peroxide and pH *abs# 67*
Joint Poster Viewing
5:00pm - 7:00pm

Evening Social - 'Party like it's the End of the World!'
7:30pm - 10:00pm
The Worldsend Hotel, 208 Hindley Street, Adelaide

Eli Lilly Dinner Meeting - Type 1 diabetes and exercise - Lessons from athletes for the clinic.
7:00pm - 9:30pm
Riverbank 2 & 3
Session sponsored by
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Are you interested in high quality, rapid turn-around, cost-effective gene manipulation services by South Australian based experts?

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• Custom cloning for basic plasmid construction
• Pilot transduction studies of customer cells using test panels of GSEx stock AAV or lentivirus

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Tuesday 25th August, 2015

Ipsen Breakfast Symposium - Neuroendocrine tumours; Diagnosis to treatment – the role of the Endocrinologist
7:00am - 8:30am

ESA Taft Lecture
8:30am - 9:30am
Chair: Warrick Inder
8:30 AM  Michael Tuttle
Is immediate surgery really necessary in every patient with primary or recurrent papillary thyroid cancer? abs# 68

SRB - Robinson Research Institute Award
8:30am - 9:10am
Chair: Sarah Robertson
8:30 AM  Mark Baker
Male Infertility: Biomarkers for the diagnosis and future prediction of men's health abs# 69

SRB - SRF Exchange Lecture
9:10am - 9:30am
Chair: Eileen McLaughlin
9:10 AM  Douglas Gibson
Do intra-uterine androgens play a critical role in preparation for pregnancy in women? abs# 70

Morning Tea
9:30am - 10:00am

ESA Clinical Symposium: Tumour profiling in endocrine cancer
10:00am - 11:30am
Chair: Ann McCormack
10:00 AM  Graeme Eisenhofer
Energy pathway metabolomics in hereditary phaeochromocytomas and paragangliomas abs# 71
10:30 AM  Roderick Clifton-Bligh
Biomarker discovery and application in Endocrine Cancers abs# 72
11:00 AM  Michael Tuttle
Tumour Profiling in Thyroid Cancer abs# 73
SRB - Newcastle Award
9:50am - 11:30am
Chairs: Brett Nixon & Shaun Roman
Session sponsored by

9:50 AM Pradeep Tanwar
Endometrial hyperplasia and cancer: Side effects of miscommunication abs# 74

10:15 AM Kirsty A Walters
Androgen Action and Ovarian Function in Health and Disease abs# 75

10:40 AM Kirsty G Pringle
Why boys are more likely to be born preterm: a novel mechanism for maintaining the fetal membranes in pregnancy abs# 76

11:05 AM Tanya Soboleva
Uncovering new roles of chromatin: control of gene activation and splicing by testis-specific histone variant, mH2A.B/H2A.Lap1. abs# 77

ESA Basic Orals - Programming and reproduction
10:00am - 11:30am
Chair: Kathryn Gatford
City Room 1 & 2

10:00 AM Nicolette A Hodyl
High circulating fetal progesterone elevates fetal free cortisol levels through cortisol displacement from corticosteroid-binding globulin abs# 78

10:15 AM Lisa K Akison
High maternal corticosterone levels during pregnancy programs sex-specific alterations in adrenal morphology and function in adult offspring of mice. abs# 79

10:30 AM Brandon R Menzies
Nutrition, growth and developmental rate affect the timing of mammalian growth axis maturation abs# 80

10:45 AM Manpreet Kaur
Restricted placental growth does not reduce spontaneous activity in the adolescent or young adult sheep. abs# 81

11:00 AM Genia Burchall
Comprehensive Assessment of the Haemostatic System in Polycystic Ovarian Syndrome abs# 82

11:15 AM Bridget Maher
Bisphenol A and childhood overweight and obesity: is there a link? abs# 83

ESA Basic Symposium: Endocrinology and ageing
10:00am - 11:30am
Chair: John Walsh & Kristy Brown
Riverbank 1

10:00 AM Roger Smith
Aging at the Start of Life. abs# 84

10:30 AM Ian M Chapman
Ageing, appetite and body composition abs# 85
11:00 AM  Roger Reddel  
Telomere biology: endocrine diseases and cancer  abs# 86

**ESA Plenary Lecture**

11:30am - 12:30pm  
Chair: Timothy Cole

11:30 AM  William Rainey  
Origins of Primary Aldosteronism  abs# 87

**SRB Orals - Meat and Livestock Award Finalists**

11:30am - 12:30pm  
Chairs: Chris Scott & Sara Edwards  
Session sponsored by

11:30 AM  Katrina J Copping  
Fetal programming in 2yo calving heifers: Effects of maternal peri-conception and first trimester protein supplementation on progeny feedlot performance, appetite and carcass characteristics  abs# 88

11:45 AM  Tamara Leahy  
Penicillamine attenuates the agglutination of ram sperm in capacitating media  abs# 89

12:00 PM  Leila Arbabi  
Y2 Receptor ligands act within the median eminence to regulate Gonadotropin Releasing Hormone (GnRH) Secretion  abs# 90

12:15 PM  Nicole A Bastian  
Regulation of Fibrillin Genes During Development of the Bovine and Human Ovary  abs# 91

**SRB - ANZPRA Award Finalists**

11:30am - 12:30pm  
City Room 1 & 2  
Session sponsored by

11:30 AM  Mancy Tong  
Detection of aggregated transthyretin in placental extracellular vesicles: importance for preeclampsia  abs# 92

11:45 AM  Rebecca Wilson  
Marginal zinc deficiency in mice during pregnancy and lactation reduces fetal growth and increases maternal blood pressure  abs# 93

12:00 PM  Rachael Crew  
Dietary-induced obesity suppresses expression of the nuclear receptor  Reverba  in placenta and fetal liver; implications for circadian and metabolic development  abs# 94

12:15 PM  Bridget Maher  
Prenatal exposure to the plasticizer bisphenol A (BPA) and adverse birth outcomes in human epidemiological studies  abs# 95
SRB Orals - Oocyte Biology
11:30am - 12:30pm
Chairs: Christopher Grupen & Karla Hutt

11:30 AM  **Laurin Lau**
NAD\(^+\) availability is critical for meiotic maturation in mouse oocytes  *abs# 96*

11:45 AM  **Wai Shan Yuen**
Plk1 is essential for establishing cortical actin polarity in mouse oocytes  *abs# 97*

12:00 PM  **Robert B Gilchrist**
Cumulin, an oocyte-secreted heterodimer of the transforming growth factor-\(\beta\) family, is a potent activator of granulosa cells and improves oocyte quality  *abs# 98*

12:15 PM  **Macarena Gonzalez**
Regulated expression of Heat Shock Proteins during ovulation and oocyte maturation.  *abs# 99*

**Lunch**
12:30pm - 1:30pm

**ESA Meet the Professor Clinical: Adverse cardio-metabolic effects of glucocorticoid therapy**
12:45pm - 1:30pm
Chair: Mark Cooper

12:45 PM  **A/Prof Morton Burt**, Department of Endocrinology, Flinders University

This conference acknowledges the sponsorship of [Lilly](#)

**ESA Meet the Professor Basic: Metabolomics**
12:45pm - 1:30pm
Chair: Frederik Steyn

12:45 PM  **Darren Saunders**
Integrated Approaches to Understanding Cancer Metabolism.  *abs# 100*

**ESA - Bryan Hudson Clinical Awards**
1:30pm - 3:30pm
Chairs: Morton Burt & Phillip Wong

1:30 PM  **Sally Abell**
Gestational diabetes mellitus and adverse pregnancy outcomes: the impact of different treatment targets at two major Australian maternity services.  *abs# 101*

1:45 PM  **Ingrid Bretherton**
Is Specificity Desirable in Urinary Free Cortisol Measurement? Comparison of 2 Immunoassays and Tandem Mass Spectroscopy.  *abs# 102*
2:00 PM  **Simon Carrivick**  
Differential associations of ferritin and 25-hydroxyvitamin D with fasting glucose and diabetes risk in community dwelling older men.  *abs# 103*

2:15 PM  **Yi Xian Chan**  
Neutral associations of testosterone, dihydrotestosterone and estradiol with fatal and non-fatal cardiovascular events, and mortality in men aged 17-97 years  *abs# 104*

2:30 PM  **Ada Cheung**  
effects of Androgen Deprivation on the Biomechanical Function of the Lower-limb Muscles during Gait in Men  *abs# 105*

2:45 PM  **Kathryn Hackman**  
Poor glycaemic control is associated with decreased survival in patients with diabetes following lung transplantation  *abs# 106*

3:00 PM  **Sonali Shah**  
The Mythology of Vitamin D Deficiency and Insufficiency  *abs# 107*

3:15 PM  **Moe Thuzar**  
Effect of Glucocorticoid on Brown Adipose Tissue Function in Humans – A Randomised Double-blind Placebo Controlled Cross-over Study  *abs# 108*

**SRB Symposium: Immune and cytokine pathways in the reproductive tract**  
1:30pm - 3:30pm  
Riverbank 2 & 3

Chairs: Brendan Waddell & Nicolette Hodyl

1:30 PM  **Jeff Keelan**  
Pharmacological strategies for the prevention of preterm birth  *abs# 109*

2:00 PM  **Martha Lappas**  
Novel targets for infection- and inflammation-induced preterm birth  *abs# 110*

2:30 PM  **John E Schjenken**  
Novel Mechanisms for Seminal Fluid Signalling in Reproduction  *abs# 111*

3:00 PM  **Ken Beagley**  
Chlamydia infections in male koalas; impacts on spermatogenesis and reproductive outcomes  *abs# 112*

**SRB Symposium: Sex Determination and Gonadal Development**  
1:30pm - 3:30pm  
City Room 1 & 2

Chairs: Ray Rodgers & Kate Loveland

1:30 PM  **Dagmar Wilhelm**  
New regulators of mammalian sex determination and gonad development  *abs# 113*

2:00 PM  **Lisa Schwanz**  
Temperature-dependent sex determination in a variable world: mechanisms of a plastic response?  *abs# 114*

2:30 PM  **Karla Hutt**  
Defining the mechanisms responsible for establishing and maintaining a pool of high quality oocytes.  *abs# 115*

3:00 PM  **Frank Grutzner**  
Monotremes provide novel insights into mammalian reproduction and disease  *abs# 116*
ESA Basic Symposium: Growth Hormone - A tale of mice and men.
1:30pm - 3:30pm Riverbank 1
Chairs: Chen Chen & Craig Harrison

1:30 PM **Vita Birzniece**
Growth hormone doping in sport *abs# 117*

2:00 PM **Andrew Brooks**
Going downstream – how does GH binding activate JAK2 *abs# 118*

2:30 PM **Frederik Steyn**
Regulation of pulsatile growth hormone secretion: Lessons from the mouse *abs# 119*

3:00 PM **Kathryn Gatford**
GH in pregnancy – how does it change and what is it doing? *abs# 120*

**Afternoon Tea**
3:30pm - 4:00pm Hall H

**ESA - Mid-Career Award Lecture**
4:00pm - 4:30pm Halls L & M
Chair: Nicolette Hodyl

4:00 PM **Lisa Moran**
Assessment and management of obesity in Polycystic Ovary Syndrome *abs# 121*

**SRB - David Healy New Investigator Award Finalists**
4:00pm - 5:30pm Riverbank 2 & 3
Chairs: James Deane & Caroline Gargett

4:00 PM **Xuan Sun**
TGFB1 is a key regulator of mammary gland macrophages during development and tumourigenesis *abs# 122*

4:15 PM **Manish Kumar**
Twists and Turns: Balanced Wnt signalling is essential for epididymal coiling. *abs# 123*

4:30 PM **Jyoti Goad**
Gone with the Wnt: Unopposed oestrogen leads to endometrial cancer by regulating Wnt signalling. *abs# 124*

4:45 PM **Siew L Wong**
Impaired embryo development due to oxidative stress can be normalised by BGP-15 treatment in vitro *abs# 125*

5:00 PM **Ella S Green**
Progesterone regulates regulatory T cell abundance and phenotype in the hormone environment of early pregnancy *abs# 126*

5:15 PM **Lisa Y.S. Lee**
The significance of aspartate in regulating blastocyst glucose metabolism *abs# 127*
ESA AGM
4:30pm - 5:30pm  Halls L & M

SRB Student Meeting
5:30pm - 6:30pm  Riverbank 2 & 3

ESA - SRB Conference Dinner
7:00pm - 10:30pm  Panorama Ballroom
Pre-dinner drinks served from 7.00pm – 7.30pm

Session sponsored by AMGEN
Wednesday 26th August, 2015

Novo Nordisk Breakfast Symposium - Mechanisms linking obesity with CV disease and Hypertension: The effects of modest weight loss.
7:00am - 8:30am
Riverbank 4
Session sponsored by

SRB Orals - The Ovarian Follicle
8:30am - 9:30am
Panorama 1,2,3
Chairs: Jock Findlay & Katja Hummitzsch

8:30 AM  Patrick Western
A common progenitor pool in the foetal ovary specifies granulosa cells of medullary and cortical ovarian follicles abs# 128

8:45 AM  Aimee Caldwell
Adverse effects of the hyperandrogenic environment on follicle dynamics in culture: a model of Polycystic Ovary Syndrome (PCOS) abs# 129

9:00 AM  Michael J Bertoldo
BMP15 and GDF9 increase intracellular cyclic AMP concentration and junctional protein mRNA expression in granulosa cells abs# 130

9:15 AM  Lisa Sercombe
mTORC1 hyperactivation induces the ovarian phenotype of PCOS abs# 131

SRB Orals - Determinants of Male Fertility
8:30am - 9:30am
Room L2
Chairs: Moira O'Bryan & Tamara Leahy

8:30 AM  David J Sharkey
Variation in Seminal Plasma Cytokine Content in Repeat Samples from Proven Fertile Men abs# 132

8:45 AM  Shaun D Roman
Alleviating the effects of acrylamide on the male germ line. abs# 133

9:00 AM  Jinghua Hu
The exposure of sperm to epididymal cysteine-rich secretory proteins (CRISPs) is required for full male fertility abs# 134

9:15 AM  Jessica Dunleavy
KATNAL2, a microtubule-regulating enzyme required for male fertility abs# 135

SRB Orals - Uterus and Endometrium
8:30am - 9:30am
Room L3
Chairs: Lois Salamonsen & Tu'uhevaha Kaitu'u-Lino

8:30 AM  Caroline E Gargett
Culture Expansion of Undifferentiated Human Endometrial Mesenchymal Stem Cells Using a Small Molecule Inhibitor abs# 136
8:45 AM  Laura A Lindsay  
The Changing Faces of ‘Receptive’ Uterine Epithelial Cells after Ovarian Hyperstimulation  abs# 137

9:00 AM  Hanan Hamimi Binti Wahid  
Toll-like receptor 4 is an essential upstream regulator of on-time parturition and perinatal viability in mice  abs# 138

9:15 AM  Florine Cynthia Martin  
Comparison and Quantitation of the uterine secretome by mass spectrometry during diapause and reactivation in the tammar wallaby  abs# 139

SRB Orals - The HPG axis
8:30am - 9:30am  Riverbank 5  
Chairs: Jeremy Smith & Linda Wu

8:30 AM  Julie-Ann P De Bond  
Altered hypothalamic metabolic gene expression in obese Kiss1r knockout mice  abs# 140

8:45 AM  Qun Li  
Expression of Kisspeptin, neurokinin B, dynorphin and GnIH prior to and after puberty in sheep  abs# 141

9:00 AM  Christopher J Scott  
Can kisspeptin treatment be used to advance/induce ovulation in anoestrous bitches?  abs# 142

9:15 AM  Alyce M Swinbourne  
Gonadotrophin releasing hormone challenge for the validation and analysis of luteinizing hormone in non-invasive urine samples from captive female southern hairy-nosed wombats (Lasiorhinus latifrons).  abs# 143

ESA Clinical Symposium: Adrenal Hormones
8:30am - 10:30am  City Room 1 & 2  
Chairs: Ray Rodgers & Venessa Tsang

8:30 AM  William Rainey  
Steroid Biomarkers for Adrenal Diseases: What’s on the Horizon?  abs# 144

9:00 AM  Mark Cooper  
In vivo measurement of cortisol metabolism  abs# 145

9:30 AM  Lucy Gagliardi  
New Concepts in Familial Bilateral Macronodular Adrenal Hyperplasia  abs# 146

10:00 AM  Graeme Eisenhofer  
Phaeochromocytoma and paraganglioma: from routine laboratory testing to disease stratification and personalised medicine  abs# 147

ESA - ADS Plenary
9:00am - 9:45am  
Chair: Timothy Davis

9:00 AM  Steven Kahn  
The Beta Cell in Type 2 Diabetes: From The Clinic to The Lab  abs# 148
SRB President’s Lecture
9:30am - 10:30am
Chair: Eileen McLaughlin
Session sponsored by Reproduction, Fertility and Development

9:30 AM Moira O’Bryan
HENMT1 Is Required for piRNA Stability and Both Male and Female Fertility. abs# 149

Morning Tea
10:30am - 11:00am
Session sponsored by SANOFI DIABETES

ESA - SRB Joint Symposium: Development programming of insulin resistance and cardiovascular disease
11:00am - 1:00pm
Chairs: Mary Wlodek & Tod Fullston
Session sponsored by novo nordisk®

11:00 AM Catherine Suter
Developmental programming of insulin resistance and diabetes abs# 150

11:30 AM Leonie Heilbronn
Understanding increased metabolic risk associated with IVF abs# 151

12:00 PM Karen Moritz
Periconceptional alcohol exposure - uterine and blastocyst contributions to offspring health abs# 152

12:30 PM Peter Mark
Metabolic and circadian perturbations in liver and adipose tissue underlie programming by glucocorticoids in rats: modulation by postnatal high fat and omega-3 fatty acids abs# 153

ESA Basic Symposium: Exercise - How much and what are the benefits?
11:00am - 1:00pm
Chair: Vita Birzniece

11:00 AM Nuala Byrne
Exercise: how much and what are the benefits for fitness versus fatness abs# 154

11:30 AM Robin Daly
Exercise prescription for bone health and fracture prevention abs# 155

12:00 PM Glenn McConell
Exercise as an intervention after IUGR abs# 156

12:30 PM David Dunstan
The Health Hazards of Too Much Sitting – What Can We Do About It? abs# 157
ESA Basic Orals - Frontiers in Endocrinology
11:00am - 1:00pm
Chair: Ann Drummond

11:00 AM  **Vincent Harley**  
DHH, ETV5 AND NEDD9 - Novel Targets of Sox9 in Mammalian Sex Determination  
*abs# 158*

11:15 AM  **Mitchell Lawrence**  
Surveying the epigenome landscape of the prostate cancer microenvironment: identification of estrogen receptor α as a key differentially methylated gene  
*abs# 159*

11:30 AM  **Heather K Armstrong**  
A novel class of Hsp90 inhibitors induce apoptosis in prostate tumours while minimising mechanisms of resistance.  
*abs# 160*

11:45 AM  **Keely M McNamara**  
Elf5 is associated with FOXA1 expression in the absence of AR and survival outcomes in triple negative cancer patients.  
*abs# 161*

12:00 PM  **Craig Harrison**  
Targeting activin to prevent muscle wasting  
*abs# 162*

12:15 PM  **Sunethra D Thomas**  
Active alternative ‘backdoor’ pathway in CAH demonstrated by urine steroid profiles  
*abs# 163*

12:30 PM  **Timothy J Cole**  
Characterization of a novel human species-restricted hydroxysteroid dehydrogenase called 11bHSD1L in the hypothalamus-pituitary-gonadal axis  
*abs# 164*

12:45 PM  **Elizabeth K Fletcher**  
Activation of the mineralocorticoid receptor promotes tissue inflammation in part via the peripheral molecular clock  
*abs# 165*

ESA Clinical Symposium: Skeletal fragility beyond BMD
11:00am - 1:00pm
City Room 1 & 2
Chairs: Roderick Clifton-Bligh & Chris White

11:00 AM  **Peter Ebeling**  
Obesity, Diabetes and Fracture Risk  
*abs# 166*

11:30 AM  **Emma Duncan**  
Osteoporosis imperfecta and other heritable disorders of bone fragility: investigation and management in adults  
*abs# 167*

12:00 PM  **Nicholas Pocock**  
Use of QCT and trabecular bone score in the assessment of Osteoporosis  
*abs# 168*

12:30 PM  **Grahame Elder**  
Renal bone disease - how to assess and manage  
*abs# 169*
ESA - ADS Joint Symposium: Oxidative Stress
11:00am - 1:00pm  Riverbank 6, 7 & 8

Chairs: Josephine Forbes & Stephen Twigg

11:00 AM  Steven Kahn
β-cell Apoptosis: The Role of Apoptosis Repressor with CARD Domain (ARC)  abs# 170

11:30 AM  Karin Jandeleit-Dahm
Reactive oxygen species and diabetic complications  abs# 171

12:00 PM  Kerry-Anne Rye
Impact of HDL on Oxidative Stress in Diabetes  abs# 172

12:30 PM  Mark Cooper
Advanced Glycated Products  abs# 173

Lunch
1:00pm - 2:00pm  Hall H

ESA Debate
1:00pm - 2:00pm  Riverbank 6, 7 & 8

ESA - SRB Orals - Hormones and Reproduction
2:00pm - 3:30pm  City Room 1 & 2

Chairs: Craig Harrison & Kirsty Walters

2:00 PM  Sarah A Marshall
Enhanced sensitivity to angiotensin II in the mesenteric arteries of late pregnant relaxin deficient mice.  abs# 174

2:15 PM  Constanze C Maresch
Testicular function and activin A in the Ins2(Akita) mouse, a model of type 1 diabetes  abs# 175

2:30 PM  Ann E Drummond
Ovarian function is regulated by the mineralocorticoid receptor  abs# 176

2:45 PM  Kelly Walton
The generation of bioactive inhibins in the absence of activins  abs# 177

3:00 PM  Carolina Soekmadji
Extracellular vesicle-mediated growth in androgen-deprived prostate cancer cells  abs# 178

3:15 PM  Gerard A Tarulli
Unraveling an identity for the androgen receptor-expressing mammary epithelial cell  abs# 179
Bioscientifica Symposium - Practical publishing advice
2:00pm - 3:30pm
Chair: Sof Andrikopoulos

2.00 PM  Introduction - Associate Professor Sof Andrikopoulos
2.05 PM  The manuscript submission process - Professor Wayne Tilley
2.15 PM  The peer review process - Dr Chris O'Nei11
2.25 PM  Responding to reviewer comments - Professor Mark Cooper
2.35 PM  Publishing ethics - Professor Ken Ho
2.45 PM  Open access - Associate Professor Warrick Inder
2.55 PM  Increasing the impact of your work - Associate Professor Ross Laybutt
3.05 PM  Question and answer session

ESA Clinical Symposium: Testosterone - The good, the bad and the ugly
2:00pm - 3:30pm
Chairs: Mathis Grossmann & Kirsty Walters

2:00 PM  Jeffrey Zajac
Androgen receptor function: The biological basis of diseases linked to testosterone abs# 180
2:30 PM  Bu B Yeap
Androgens and cardiovascular risk abs# 181
3:00 PM  David Handelsman
Andropause/Low T: The Masquerade of Sick Eugonadism abs# 182

ESA Basic Orals - Metabolic
2:00pm - 3:30pm

2:00 PM  Mojgan Nazari
Using translating ribosome affinity purification (TRAP) to investigate gene expression in beige or ‘browned’ adipocytes abs# 183
2:15 PM  Helen L Barrett
Expression of hexosamine signaling pathway genes in placentae from women with gestational diabetes mellitus (GDM) abs# 184
2:30 PM  Rajes Qvist
Beta Adrenergic receptors stimulation attenuates hyperglycemia-induced inflammation and apoptosis via NF-κB and IκBα in endothelial cells abs# 185
2:45 PM  Albert Wu
Age-related changes in estradiol and longitudinal associations with fat mass in men abs# 186
3:00 PM  Mary Wlodek
Metabolic and fetal benefits of endurance exercise training for females born small on high fat diet abs# 187
3:15 PM  Anjana Radhakutty
Effect of low dose glucocorticoid therapy on arginine metabolism in patients with rheumatoid arthritis abs# 188
ESA Clinical Orals - Pituitary and Adrenal
2:00pm - 3:30pm Room L1
Chair: Mark McLean

2:00 PM **Warrick Inder**
Thrombospondin-1 is a glucocorticoid responsive protein and potential biomarker of glucocorticoid activity. *abs# 189*

2:15 PM **Katherine English**
The role of a day 5 metyrapone test in assessing the HPA axis post pituitary surgery, a prospective trial *abs# 190*

2:30 PM **Anthony R Lam**
Loss-of-function germline *FGFR1* mutation identified in a patient with prolactinoma *abs# 191*

2:45 PM **Viral Chikani**
Growth hormone replacement improves anaerobic capacity and physical function in adults with growth hormone deficiency *abs# 192*

3:00 PM **Jessica E Harris**
Germline mutation in the MET proto-oncogene, receptor tyrosine kinase/hepatocyte growth factor receptor (*MET*) in a patient with phaeochromocytoma – a new gene for this disorder *abs# 193*

3:15 PM **Nicolette A Hodyl**
A larger cortisol awakening response is associated with improved later day cognitive function *abs# 194*

ESA Clinical Orals - Clinical Highlights 2015
2:00pm - 3:30pm Room L3
Chair: Don McLeod

2:00 PM **John P Walsh**
Reconciling the log-linear and non-linear aspects of the TSH-free T4 relationship: intra-individual analysis of a large population *abs# 195*

2:15 PM **Emma Scott**
Endocrinopathies associated with immune modulation therapy for the treatment of metastatic melanoma *abs# 196*

2:30 PM **Alexander J Rodriguez**
Association between plasma adipocytokine concentrations and microvascular complications in patients with type 2 diabetes mellitus: a systematic review and meta-analysis of controlled cross-sectional studies *abs# 197*

2:45 PM **Jessie Teng**
Effect of adrenocorticotropic hormone stimulation on the outcomes of adrenal vein sampling in primary aldosteronism *abs# 198*

3:00 PM **Stefan Bagheri-Fam**
Ligand-independent activation of FGFR2c leads to XY sex reversal in humans and mice *abs# 199*

3:15 PM **Radhika Seimon**
Fast weight loss does not reduce muscle strength or bone mineral density compared with slow weight loss in obese post-menopausal women *abs# 200*
SRB Workshop - Doctors without academic borders - alternate PhD career paths

2:00pm - 5:00pm
Chairs: John Schjenken & Tamara Leahy

2:00 PM  **Simon Barry**  
A/Prof Simon Barry, University of Adelaide  *abs# 201*

2:20 PM  **Leigh Guerin**  
Dr Leigh Guerin, Phillips Ormonde Fitzpatrick  *abs# 202*

2:40 PM  **Melanie Bagg**  
Dr Melanie Bagg, The Australian Science Media Centre  *abs# 203*

3:00 PM  **Ryan Rose**  
Ryan Rose, Fertility SA  *abs# 204*

3:20 PM  **Kiri Beilby**  
Kiri Beilby, Origio  *abs# 205*

ESASenior Plenary

3:30pm - 4:30pm
Chairs: Peter Ebeling

3:30 PM  **Evan Simpson**  
Oestrogen – the good, the bad, and the unexpected  *abs# 333*
Prevent the first break from happening. And you could prevent a second.

Recommend a bone scan when your patients turn 70 and you could prevent the first break from ever happening.

Fracture protection within 6 months1

1 Across vertebral and non-vertebral fractures.

**PBS Information:** Actonel EC. Restricted Benefit. Actonel EC Combi, Actonel EC Combi D. Authority Required (STREAMLINED). Refer to PBS schedule for full restricted authority information.

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**ACTONEL EC, ACTONEL EC COMBI & ACTONEL EC COMBI D. Presentations:** Actonel EC 35mg tablets contain 35mg of risendronate sodium. Actonel EC Combi: 1 Actonel EC 35mg tablet and 6 calcium carbonate tablets 1250mg (equivalent to 500mg elemental calcium) tablets. Actonel EC Combi D: 1 Actonel 35mg tablet and 6 daily sachets containing 2500mg calcium carbonate and 22ug cholecalciferol, equivalent to 1000mg elemental calcium and 22ug (880IU) vitamin D3 respectively. **Indications:** Actonel EC 35mg tablet, Actonel EC Combi, Actonel EC Combi D. Treatment of osteoporosis. Treatment of glucocorticoid-induced osteoporosis. Preservation of bone mineral density in patients on long term corticosteroid therapy. **Contraindications:** Risendronate: Hypersensitivity to the drug or ingredients, hypocalcaemia, inability to stay or stand upright for at least 30 minutes. Calcium carbonate: Hypersensitivity to the drug or ingredients, hypercalcemia, hypercalciuria, nephrolithiasis. Cholecalciferol: Hypercalcemia, hypercalciuria, nephrolithiasis, hypervitaminosis D. **Precautions:** Risendronate: Hypocalcaemia, bone and mineral metabolism dysfunction; calcium and vitamin D if dietary intake is inadequate; severe renal impairment; peptic ulcer disease; inflammatory bowel disease; osteonecrosis of the jaw; dental examination with preventive dentistry; avoid invasive dental procedures; atypical stress fractures; pregnancy (Category B3): certain medications (e.g. calcium supplements, antacids). Calcium carbonate and/or Cholecalciferol: Impairment of renal function; monitoring of serum calcium levels and renal function; other drugs containing vitamin D, sarcopenia; immobilised patients due to the increased risk of hypercalcemia, increased absorption of calcium, malabsorption, dehydration. **Adverse Effects:** Risendronate: Common: abdominal and musculoskeletal pain. Uncommon: glossitis, iritis, and duodenitis, abnormal liver function tests. Postmarketing: varied; hypersensitivity and skin reactions, including angioedema, generalised rash and bullous skin reaction, urticaria and osteonecrosis of the jaw. See full PI. Calcium carbonate and/or Cholecalciferol: Uncommon: hypercalcemia, hypercalciuria. Rare: flatulence, constipation, nausea, abdominal pain, diarrhoea, pruritus, rash and urticaria. **Dosage and Administration:** Actonel EC 35mg tablets: take with a glass of plain water either with or without food once a week taken on the same day each week. Actonel EC should be taken in an upright position. Patients should avoid lying down for 30 minutes. Tablets must be swallowed whole. Actonel EC Combi: Two component therapy consisting of 7 tablets in a blister, 1 Actonel EC 35mg tablet and 6 calcium carbonate tablets. The recommended dose in adults is 1 Actonel EC 35mg tablet on the first day, followed by, beginning on the next day, 1 calcium carbonate sachet daily for 5 days. This 7 day sequence is then repeated each week. Actonel EC Combi D: Intended for patients for whom the amount of calcium and cholecalciferol included is considered to provide adequate supplementation. The recommended dose is 1 Actonel EC 35mg tablet on the first day, followed by, beginning on the next day, 1 calcium carbonate/cholecalciferol sachet daily for 5 days. This 7 day sequence is then repeated each week starting with the Actonel EC 35mg tablet. Date of most recent amendment February 2015. **Name and Address of the Sponsor:** Actavis Pty Ltd, 5/f, 1117 Harrington St, The Rocks, NSW 2000

**References:**

Allergan Pty Ltd. 610 Pacific Highway, Gordon NSW 2072, Australia. Date prepared: July 2015, AU/0252/2015. ADworks 12204
ESA-SRB Poster Listing

ESA Poster Session: Basic Science

Ki Yong Chung
Effects of dietary probiotic on growth performance, blood characteristics, and immune responses to a lipopolysaccharide challenge of Hanwoo heifers abs# 206

Amy Hsieh
Endocrine collateral damage abs# 207

Katharine E Johnson
Biological Activity and In Vivo Half-Life of Pro-Activin A abs# 208

James R McFarlane
Pharmacokinetics of Leptin in the Gut of Mice abs# 209

James McFarlane
Is oxytocin receptor SNP rs53576 a potential biomarker for psychological resilience? abs# 210

Makoto Ono
FGF9 activity from normal males and a 46,XY female abs# 211

Christopher J Scott
Castration Effects on the Expression of Kisspeptin and Rf-Amide Related Peptide-3 and their Co-Expression with Oestrogen Receptor a in the Ram Hypothalamus. abs# 212

Mary Wlodek
F2 fetal nephron number and weight benefits of endurance exercise training for females born small on high fat diet abs# 213

Veronica Wong
Pituitary Metastases abs# 214

ESA Poster Session: Clinical

Aleena S Ali
Management of Diabetes in Lung Transplant Recipients abs# 215

Imran Badshah
Time-specific basal cortisol cut-offs are a more reliable predictor of passing a Synacthen Stimulation Test than a single threshold level. abs# 216

Vita Birzniece
Tamoxifen reduces hepatic VLDL production in women: a possible GH-mediated mechanism for the development of fatty liver abs# 217

Ramy Bishay
Hypophosphataemic osteomalacia associated with iron infusions: Report of three cases abs# 218

Ramy Bishay
Extremes of autoimmune thyroid dysfunction associated with interferon treatment in one patient abs# 219

Kharis Burns
Iodine status in women of childbearing age abs# 220

Ada Cheung
Increased fat mass contributes to increased insulin resistance in men undergoing androgen deprivation therapy for prostate cancer. abs# 221
Ian m Collins
Ovarian Reserve of Women with Germline BRCA1 or BRCA2 Mutations. abs# 222

Casey de Rooy
Histological skeletal muscle changes in men with prostate cancer undergoing androgen deprivation therapy. abs# 223

Casey de Rooy
Quality of life decrements in men with prostate cancer undergoing androgen deprivation therapy. abs# 224

Sunita MC De Sousa
Comparison of the insulin tolerance test against the glucagon stimulation and short Synacthen tests in patients with suspected hypopituitarism. abs# 225

Chris Gilfillan
Fine needle aspiration of the thyroid: correlation with final histopathology in a series of 187 patients. abs# 226

Florence Gunawan
Transient Hypercalcaemia in Hospitalised Elderly Patients: an Association with Underlyng Hyperparathyroidism and Vitamin D Supplementation abs# 227

Hui Yi HN Ng
Timely Commencement of Anti-resorptive Therapy Post Fragility Fractures: a Discrepancy Between Recommendations and Clinical Practice abs# 228

Julie Hetherington
“Parachutes to Prevention” – A conceptual change in acute adrenal insufficiency education abs# 229

Michelle Isaacs
A case of primary amenorrhoea and hyperandrogenism abs# 230

Michelle Isaacs
An Odd Hot Spot abs# 231

Tripti Joshi
Calcium stimulation test to localize insulinomas- Local centre experience abs# 232

Min Sun Kim
IGF-1/IGFBP-1 axis are closely associated with insulin secretion in Korean children. abs# 233

Elena R Kornaczewski
Utility of FDG-PET CT scanning in succinate dehydrogenase B mutation related lesions abs# 234

Melissa H Lee
Hypokalaemia Post-Saline Suppression Test in Primary Hyperaldosteronism abs# 235

Melissa Lee
Dilemmas In The Diagnosis Of Cushing’s Syndrome In The Acutely Unwell Patient abs# 236

Jaime Lin
Treatment Resistant Papillary Thyroid Cancer abs# 237

Shao Feng Mok
Subclinical hypothyroidism in pregnancy related to TSH receptor blocking antibodies: An unique clinical conundrum abs# 238

N Shankara Narayana
Accuracy of Direct Progesterone Immunoassay vs Liquid Chromatography Mass Spectrometry abs# 239
Marni A Nenke
Paradoxical reduction in corticosteroid-binding globulin cleavage is seen in alpha-1 antitrypsin deficiency: implications for cortisol homeostasis abs# 240

Luisa Olaya
The Prevalence of BRAF V600 Mutations and its Associated Histopathology Features in Papillary Thyroid Carcinoma in New Caledonia and Australia abs# 241

Karl Peters
Effect of denosumab on glucose control in subjects with diabetes or pre-diabetes from the FREEDOM study abs# 243

Jeyakantha Ratnasingam
Predictors For Surgically Resected Non-Functioning Pituitary Adenoma Requiring Secondary Intervention abs# 244

Emma Scott
Characteristics, Diagnoses and Clinical and Genetic Outcomes of Patient Population Attending a Multidisciplinary Familial Endocrine Neoplasia Clinic abs# 245

Radhika V Seimon
Intermittent moderate energy restriction improves weight loss efficiency in diet-induced obese mice abs# 246

Divya Srivastava
Radioactive Iodine ablation of differentiated Thyroid cancer as per 2009 ATA guidelines and future directions: single centre experience –retrospective review. abs# 247

Thomas Upton
Acromegaly: Outcomes from a single pituitary surgeon service in Christchurch New Zealand abs# 248

Anna K Watts
Graves’ Dermopathy: a report of three cases abs# 249

Anna Watts
Regrowth of non-functioning pituitary macroadenomas undergoing surgery in a single Australian centre. abs# 250

Nisha Venkatesh
Examining the indications and results of bone densitometry performed in a large metropolitan teaching hospital. abs# 251

Jasmine J Zhu
Post-Partum Osteoporosis Due To Systemic Mastocytosis: 2 Case Studies abs# 252

ESA Poster Session: Clinical Case Study

Dushyanthy Arumugam
Hurt in the Sternum abs# 253

Kharis Burns
Desmopressin, Oxytocin and a Failing Heart abs# 254

Daniela Chan
A rare type of aggressive thyroid cancer : review of the literature for treatment options abs# 255

Anna Galligan
Undiagnosed Asymptomatic Phaeochromocytoma Causing Intra- Operative Haemodynamic Crisis in a Patient with Type One Diabetes. abs# 256
**Rinky Giri**
Hypoglycemic Encephalopathy and the Severity of Brain Injury: A Case Report  *abs# 257*

**Thomas Hadwen**
A case of frontal bone aneurysmal bone cyst in association with polyostotic fibrous dysplasia  *abs# 258*

**Jessica E Harris**
MEN1 and paraganglioma: expanding the clinical spectrum of MENIN mutations.  *abs# 259*

**Brianna Hatswell**
Tetany Associated with Teriparatide Therapy: A Case Report  *abs# 260*

**Alice Hong**
Bilateral macronodular adrenal hyperplasia and systematic testing for aberrant receptors: a bumpy journey  *abs# 261*

**P M Jansen**
Multiple paragangliomas in a 17-year old male with post-micturition symptoms  *abs# 262*

**Pieter M Jansen**
Hemiballismus: a rare complication of diabetic nonketotic hyperosmolar state  *abs# 263*

**Angela S Lee**
Euglycaemic diabetic ketoacidosis in a young adult with type 1 diabetes and an eating disorder  *abs# 264*

**Melissa H Lee**
Double Trouble In The Pituitary: A Case Report  *abs# 265*

**Kristina McDonnell**
An unusual cause of recurrent severe hypokalaemia  *abs# 266*

**Emily Meyer**
*Pituitarius*, where art thou?  *abs# 267*

**Tara Naige**
Flushed with excitement – a heartfelt case of carcinoid syndrome  *abs# 268*

**Anna K Watts**
Thyroid hormone resistance, a case report  *abs# 269*

**Sharon Yeoh**
Vitamin C deficiency: an overlooked risk factor for impaired wound healing in patients with diabetes mellitus  *abs# 271*

**SRB Poster Session: Embryogenesis, Assisted Reproductive Technology and Stem Cells**

**Daniel Barry**
Development of Ovine and Murine Embryos Under Micro-Gravity Conditions at the International Space Station  *abs# 272*

**Cassandra O. Carbone**
Addition of a Mitochondrial Antioxidant to Culture Media Improves Embryo Development and Metabolism in an Aged Mouse Model  *abs# 273*

**Miaoxin Chen**
Decreased antioxidative gene expression in skeletal muscle in individuals conceived by In-Vitro Fertilisation (IVF)  *abs# 274*
Sangrae Cho
Efficacy of a Cue-Mate Intravaginal Insert and Injection of Prostaglandin F₂α For Synchronizing Estrus in Hanwoo Cattle abs# 275

Suyinn Chong
Epigenetic and microRNA-mediated regulation of adult hippocampal VGLUT2 expression following early prenatal ethanol exposure abs# 276

Sara J Edwards
Factors contributing to the poor reproductive performance of ewe lambs abs# 277

Consuelo Estrella
Differential maternal and paternal genome effects on circulating thyroid hormone concentrations and deiodinase expression in the midgestation fetus abs# 278

Rebecca Kelley
Consequences of culturing preimplantation embryos individually abs# 279

Yan Li
Effect of DNA methyltransferases on the reprogramming of methylation during early mouse embryo development abs# 280

Mark B Nottle
Effect of thyroid hormones on porcine oocyte maturation in vitro. abs# 281

Mark B Nottle
Multipotent cell types in primary fibroblast cell lines used to clone pigs using somatic cell nuclear transfer. abs# 282

Sangho Roh
Thiazovivin, a Rho kinase inhibitor, improves stemness maintenance of bovine embryo-derived pluripotent stem cells under chemically defined culture conditions abs# 283

Cheow Yuen (Tiffany) Tan
Grey Level Co-occurrence Matrix (GLCM): A novel method to access the texture of mouse embryos derived from assisted reproductive technology (ART) abs# 284

Emma K Tregoweth
Hyperglycaemic stress increases blastomere heterogeneity in pre-implantation mouse embryos abs# 285

Jiayi Wan
The reduction in melatonin level may contribute to the pathogenesis of ovarian cancer abs# 286

SRB Poster Session: Growth factors/Cytokines/Immune system

Christina D Marth
The influence of ovarian hormones on the uterine immune response abs# 287

Nour Nicolas
Testicular activin A during the development of autoimmune orchitis in mice abs# 288

Michael W Pankhurst
Anti-Müllerian Hormone (AMH) has an increased rate of conversion to the active form after puberty. abs# 289

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Michael J Bertoldo
In Utero Exposure to the Insulin Sensitising Drug Metformin Reduces the Fertility of Male Offspring abs# 291
Yu Chen
Transcriptome analysis of the developing phallus after hormonal manipulation in a marsupial abs# 292

Mariana M Neves
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Booth 16
Web: www.endocrinesociety.org.au
Ph: 02 9256 5405, Fax: 02 9251 8174
Contact: Ivone Johnson, Executive Officer
Email: ijohnson@endocrinesociety.org.au
The Endocrine Society of Australia (ESA) 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 ten members of its Council who are elected every two years by a ballot of the membership in accordance with the Constitution.

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Contact: societyreproductivebiology@gmail.com
The Society for Reproductive Biology fosters and promotes the advancement and dissemination of basic and applied research in reproduction, fertility and development directed towards improving biomedicine, health, agriculture and conservation. It is the premier society for scientific research in reproductive biology in Australasia and currently encompasses more than 300 members.
Evolution within the mitochondria – adaptation, conflict, and the health implications

Damian Dowling

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Mitochondria are essential to eukaryotes, producing most of the cellular energy requirements. Curiously, they have retained their own diminutive genome – of mitochondrial DNA (mtDNA); which is maternally inherited, and encodes a handful of proteins that comprise subunits of the mitochondrial electron transport chain. Biologists long reasoned that natural selection would prevent any phenotype-modifying genetic variation from accumulating within the mitochondrial genome, given that the products of the mtDNA are so critical to life. I will present research that challenges this traditional paradigm – by showing that mtDNA sequences typically harbour genetic polymorphisms that affect metabolic and reproductive health, and life expectancy. I will present experimental evidence from natural populations of fruit fly (Drosophila melanogaster) that some of the polymorphisms that delineate the mtDNA haplotypes of different geographic regions are adaptive and have evolved under natural selection to the local climate. I will show that the effects of mtDNA haplotypes on health are often contingent on the nuclear background. That is, the particular combination of mtDNA and nuclear genes an individual harbours (the joint “mito-nuclear” genotype) is an important predictor of that individual's reproductive prospects and life expectancy. I will then outline the evolutionary consequences of maternal inheritance of mtDNA for male health. Maternal inheritance means that male-harming mutations can accumulate in the mtDNA sequence. If these same mutations are relatively benign, or even beneficial, in their effects on females, then in theory natural selection will fail to eliminate them (since all of the screening of mtDNA mutations is done directly through females). Thus male-harming, but female-friendly, mutations are predicted to accumulate within the mitochondrial genomes of animals, and affect male health components. I will present our research in fruit flies, which substantiates this evolutionary theory.

Males deliver more than DNA

Angela Crean

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Modern evidence of non-genetic inheritance mechanisms that operate alongside and in conjunction with genetic inheritance has revolutionized our understanding of how variation is transmitted across generations. Maternal effects have long been recognised as having important non-genetic influences on offspring phenotype. In contrast, males are often considered to contribute ‘nothing but sperm’ to reproduction. A sperm’s job is to deliver DNA to an egg, and DNA is not influenced by the environment. Hence, it was thought that the paternal environment could not influence offspring traits. Using two very different study systems – sea squirts and neriid flies – I have empirically demonstrated that plasticity in sperm and semen quality can influence offspring growth and survival, even in species that provide ‘nothing but sperm’. Observed patterns of inheritance suggest that different mechanisms are driving non-genetic paternal effects in each system. Sea squirts reproduce by releasing both eggs and sperm into the ocean where fertilization occurs, making them a very tractable system to examine links between sperm plasticity and offspring phenotype. I found that males in high density environments make more competitive sperm (increasing fertilization success) at a cost to successful offspring development. However, post-settlement, offspring survive better when their environment matches their fathers, suggesting that males may be able to prepare their offspring for future environmental challenges. Neriid flies mate like the majority of land animals, but fly eggs are not fertilized until immediately before they are laid. This allowed me to decouple exposure to seminal fluid from fertilization, showing that the diet of a mating partner who does not sire offspring can influence the size of future offspring sired by another male. Not only does this remarkable effect change the way we view inheritance, it also tells us that non-sperm components of the seminal fluid can mediate non-genetic paternal effects.

Establishing epigenetic change as the mediator of fetal programming in humans: are we there yet?

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The Developmental Origins of Health and Disease (DOHaD) hypothesis predicts that environmental exposures experienced early in life modify risk associated with later onset disease. DOHaD is supported by a large number of direct animal studies and a smaller number of compelling observational studies in humans, but the mechanism(s) underlying the 'programming' of DOHaD effects remain largely unclear. Epigenetic variation has rapidly emerged as a candidate mediator of such effects. However, little direct evidence exists in humans, primarily due to the inherent problems associated with unraveling the relative contributions of genetic and environmental influence to phenotypic outcome. Our team aims to address some of these knowledge gaps through the establishment of longitudinal human cohorts of varying design, commencing prior to birth, with detailed environmental, clinical and other data, and extensive biospecimen collection. Such studies are key to providing direct evidence in support of a role of epigenetic processes as a driver of DOHaD in humans.

Estrogen paradox: how can estrogen both cause and prevent breast cancer?

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Epidemiological studies provide strong evidence that estrogens contribute to the development of de novo breast cancer and to their growth when established. Specifically, these studies demonstrate an increased risk of breast cancer with early menarche, delayed first birth, late menopause, high body mass index, breast density, plasma estrogen levels, and use of menopausal hormone therapy (MHT) containing estrogen plus a progestogen. Paradoxically, estrogen therapy alone in the Women’s Health Initiative (WHI) study reduced breast cancer risk by 23% (HR 0.77, 95% CI 0.62-0.95) at 13 years of follow-up (7 years of therapy and 6 years without). The mechanisms for this paradox are becoming better understood and will be reviewed in the presentation. Estrogens appear to cause de novo breast cancer by both ERα-dependent and - independent effects. ERα-dependent actions result in an increase in the rate of cell division and resulting mutations. By the conversion to genotoxic metabolites, estrogen causes mutations in an ERα–dependent fashion. The ERα knock out /Wnt 1 transgenic mouse model provides the most compelling evidence of both ERα-dependent and -independent (i.e genotoxic) effects. As evidence of ERα-dependent effects, knock out of ERα reduces the incidence of breast cancer. Supporting an ERα-independent effect, reduction of estradiol by oophorectomy in ERα knock out animals reduces the incidence of breast cancer and add back of this sex steroid restores this parameter. Blockade of any residual ERα or β activity with the "pure" antiestrogen fulvestrant did not alter these findings. Clinical studies also support an ERα-independent mechanism as oophorectomy reduces the incidence of ER negative breast cancer in women with BRCA1 mutations. Importantly, the BRCA1 mutation is associated with increased production of genotoxic metabolites in women.

To understand how estradiol paradoxically prevents breast cancer, we developed two models to describe the life history of breast cancer and validated these in 7 population models. It takes an average of 16 years to proceed from development of de novo cancer to reach the detection threshold of mammography or MRI. At autopsy, 7% of normal women ages 40-80 harbor occult tumors too small to be detected by imaging. Accordingly, MHT effects in the WHI largely related to occult, established tumors. The average age of women receiving estrogen alone in the WHI was 63 and these women had undergone a period of estrogen deprivation of 12 years on average after menopause. Based on our data, the reduction of breast cancer with estrogen alone in the WHO appears likely to have resulted from estradiol-induced apoptosis. As evidence, our in vitro model of long term estradiol deprivation demonstrated that estradiol causes apoptosis via both death receptor and mitochondrial pathways. A key regulatory factor is AMP-K with its downstream pro-apoptotic effects on FOXO-3, FAS-1, GADD 45, and BIM.

In summary, estradiol can cause de novo breast cancer through ERα-dependent and- independent effects and cause cell death in established, occult tumors in post-menopausal women receiving estrogen alone many years after onset of menopause.

Alternative splicing of HOX genes and their adjacent long noncoding RNAs may regulate sexually dimorphic phallus development

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Hox genes are evolutionarily highly conserved, and regulate a network of genes controlling development of body plan. In particular, the role of Hox genes in formation of the appendages, namely the phallus and limbs, appear very similar. Regulation
of Hox genes during appendage development involves IncRNAs. However, how HOX and adjacent IncRNAs regulate development of the phallus are largely unknown. In marsupials, development of the phallus shows distinct differences in timing relative to the corresponding events in eutherian mammals. The neonatal phallus is identical from birth until after day 50 post partum (pp) when gene expression changes markedly and when male phallus growth accelerates. The morphological differentiation of the male phallus is modulated by androgen exposure between d 20-30 pp. In tammar, we can induce sex reversal of the urogenital organs, including the phallus. We therefore induced male-type phallus development in developing females by exposure to androgens (androstanediol) from day 25-30 and day 25-50 and induced hypospadias in developing males by castration at day 25 or by oestrogen treatment from birth to days 30 and 50. Phallic tissues were collected at day 30 and day 50 pp for RNA-Seq after these treatments. We focussed on the HOX genes and adjacent IncRNAs with RNA-Seq data and compared these to Chip-Seq data of human and mouse. There was more alternative splicing than expected, even for the highly conserved Hox genes. After analysis of all Hox genes and adjacent IncRNAs, we showed that while transcription levels of HoxD13 are not different between phallus tissues, the isoforms expressed were markedly different between male and females. Our findings were confirmed with specific qPCR and miRNA in situ hybridization. In conclusion, our data suggests that alternative splicing of key genes, such as Hox and their adjacent IncRNAs may regulate the development of the phallus.

6

Paternal miR146a influences the female post-coital inflammatory response to seminal fluid

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Exposure to seminal fluid at coitus induces the expression of cytokines and chemokines in the female reproductive tract, which activates and recruits immune cells including dendritic cells, macrophages and T-regulatory (Treg) cells. These cells are crucial for implantation and the establishment of feto-maternal immune tolerance. Signalling factors in seminal fluid, including transforming growth factor beta (TGFβ) and prostaglandin-E (PGE) have been identified but studies suggest there are, as yet unidentified signalling molecules. Our recent studies have suggested that microRNAs (miRNAs) may contribute to the seminal fluid signalling cascade. One miRNA of interest, miR146a, is induced post-coitus and is carried by sperm. This miRNA regulates Treg cell functions, targets the Nfκb inflammatory pathway and may contribute to spermatogonial differentiation and PGE production. We hypothesised that sperm derived miR146a is delivered to the female tract at coitus and influences the post-coital inflammatory response. The expression levels of miR146a and seminal fluid-induced cytokines were examined by qPCR in the endometrium of unmated estrus CBA x C57Bl/6 F1 (CBAF1) female mice, or CBAF1 females 8 hours after mating with either miR146a+ or miR146a− males (n=9-12 per group). Paternal miR146a deficiency did not alter miR146a expression in the endometrium following coitus, compared with miR146a+ mated group. Despite this, the absence of paternal miR146a resulted in a significant decrease in the expression of the key peri-conception cytokines Csf2, Csf3, Il1b, Il6, Tnfa and Il1f and a significant increase in Ccl21a and Il10 expression compared to miR146a+ mated females. These findings demonstrate that while paternal miR146a is not delivered to the female tract at coitus, the absence of this miRNA in seminal fluid may alter the post-coital inflammatory response. Future studies are required to determine how cytokines are regulated by paternal miR146a and whether paternal miR146a deficiency may lead to altered pregnancy outcomes and offspring development.

7

Seminal Fluid Regulates miR155, which Impacts on Treg Cells and Alters Pregnancy Outcomes

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Maternal immune tolerance of the semi-allogeneic fetus requires CD4+FOXP3+ T-regulatory (Treg) cells, which suppress inflammation and anti-fetal immunity. Expansion of the Treg cell pool in preparation for conception is mediated by seminal fluid. Recent studies have demonstrated that microRNAs contribute to Treg cell function with miR155 established as a key Treg cell miRNA. However, the physiological importance of miR155 in early pregnancy and Treg expansion, and whether miR155 contributes to pregnancy success is unknown.

CBA x C57Bl/6 F1 female mice (n=8-10) were mated with Balb/c males and sacrificed at 8, 16, 36, 60 and 84h post-coitus (pc). Uterine-draining lymph nodes (PALN) were collected and miRNA expression levels were determined using qPCR. miR155+/− or wildtype (WT) females (n=11-13) were mated with Balb/c males and sacrificed on day 3.5 pc. The percentage of Treg cells in the mesenteric lymph nodes (mLN), spleen, blood and PALN was examined by flow cytometry. Pregnant miR155+/− or WT (n=20-23) females were sacrificed on day 17.5pc, fetal and placental weights were determined to assess pregnancy outcome.

Exposure to seminal fluid suppressed miR155 expression in PALN throughout the peri-conception period with the most significant reduction (2.5-fold) observed 8h pc. miR155 deficiency resulted in a systemic reduction in the percentage of Treg cells compared to the WT control with the most significant reduction being observed in the PALN (2.6-fold). Maternal miR155 deficiency altered the outcomes of pregnancy with a 6.0% increase in fetal weight and 7.9% increase in the fetal:placental ratio in late gestation.
In conclusion, seminal fluid suppresses miR155 during early pregnancy and the absence of miR155 is associated with reduced Treg cell proportions, and may lead to altered fetal and placental development. These data indicate a potentially important role for miR155 in the expansion of Treg cells during early pregnancy, and may provide novel diagnostic therapies for Treg-associated pregnancy pathologies.

Potential effect of activin on establishment of piRNA machinery in human germ cells

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The growth factor activin functions in testis development at multiple stages that influence the pace of testis development and germ cell differentiation. Activin levels become highly elevated in the fetal testis where its activity regulates gonocyte (male germ cell) and Sertoli cell proliferation. This elevation occurs in a critical period for renewal of DNA methylation in gonocytes, and we considered activin as a candidate for regulating the piRNA machinery that protects the unmethylated gonocyte genome. Piwi-interacting RNAs (piRNA) are crucial for spermatogenesis because they repress activation of transposable elements via recruitment of DNTM3, which catalyzes protective de novo methylation on genomic DNA. In mice, deletion of piRNA synthesis machinery in knockouts results in male-specific sterility; these germ cells exhibit aberrant LINE-1 transposon activation, morphologically abnormal chromatoid bodies in spermatocytes (which also synthesize piRNA) and eventual absence of spermatogenesis in adults. However, it remains unclear how the comprehensive activation of genes mediating piRNA activities is achieved. We evaluated activin regulation of piRNA machinery using a human seminoma cell line, TCam-2, derived from a germ cell tumour that is most closely related to gonocytes.

TCam-2 cells were cultured with activin A (5 ng/ml) plus minus 10% fetal calf serum for 24 hours. Real time RT-PCR (three experiments; n=3 per experiment) was conducted to quantify transcripts relating to piRNA machinery. Amongst the PIWI, TDRD and DNTM3 protein families, activin A significantly downregulated DNTM3 and TDRD1 which are both male-specific in fetal germ cells, as well as another marker, NANOS2. In contrast, TDRD5 and TDRD7, which are required for chromatoid body assembly in spermatocytes, were up-regulated by activin A, but only under conditions of serum starvation. We propose that activin A influences establishment of piRNA machinery in fetal male germ cells and contributes to chromatoid body assembly via controlling TDRD5 and TDRD7 production during spermatogenesis.

A paternal high fat diet alters founder sperm microRNA profile and implicates it as part of a candidate epigenetic mechanism underlying paternal programing.

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Obesity and related comorbidities are increasingly globally prevalent. We have previously demonstrated that a paternal high fat diet (HFD) programs two subsequent generations of mice for reproductive and metabolic dysfunction.

We now demonstrate that a paternal HFD concomitantly shifts the microRNA profile of sperm. 28 sperm borne microRNAs were initially indicated as being differentially abundant by qPCR array card (n=4 per CD/HFD). Subsequently 13/28 microRNAs were confirmed as differentially abundant in sperm from three separate cohorts of mice fed a HFD by individual qPCR assays (n=13 CD/14 HFD). Interestingly 2/13 of these microRNAs were: (i) upregulated by the HFD; (ii) are presumed sperm specific (not detectable in oocytes, present in zygotes) and (iii) their homologs are amongst the most abundant microRNAs in human sperm. Experimentally validated, developmentally important mRNA targets (Oct4, Sox2) of one of the sperm specific microRNAs have altered expression in zygotes sired by HFD males.

Preliminary analysis suggests that the same microRNAs might also be dysregulated in sperm of offspring from HFD fed founder males, albeit in an inverse direction and despite being separated by a chasm of developmental time.

HFD fed founder sperm microRNA content appears to be somewhat restored by interventions that target obesity (diet and/or exercise). This partial restoration of sperm microRNA content occurs with partial restoration of subfertility and metabolic phenotypes in offspring born to HFD fed founders.

Overall this suggests sperm borne microRNAs form part of an epigenetic mechanism, sensitive to the dietary/metabolic state of a male, capable of having molecular consequences in the preimplantation embryo. This potentially triggers a molecular cascade that impairs embryo development and programs the F1 generation for reproductive and metabolic dysfunction. Furthermore this information may provide an under-recognised window of opportunity for intervention that holds the potential to improve the health of the future generations.
RBM5 is required for spermatogonia differentiation
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Balance of spermatogonial stem and progenitor cell (SSPC) self-renewal and differentiation is essential for the homeostasis of spermatogenesis and the maintenance of male fertility. Regulation of SSPC function requires a complex interplay of intrinsic and extrinsic niche-derived factors. In this study, we identified the splicing factor RBM5 as a novel regulator of spermatogonia differentiation. Male mice carrying an ENU-induced missense mutation (R263P) in the second RNA recognition motif (RRM2) of RBM5 were sterile due to a round spermatid arrest, which ultimately led to azoospernia. We have shown that RBM5 is an essential splicing factor in round spermatids and the R263P mutation resulted in aberrant splicing in several target pre-mRNAs that are required for spermatid differentiation. Within the adult mouse testis, RBM5 localises to the nucleus of somatic and germ cells including spermatogonia, spermatocytes and round spermatids. Further a stereological analysis revealed that in addition to the spermatid arrest phenotype Rbm5 mutant mice have a decreased conversion of spermatogonia into spermatocytes and significant loss of late spermatocytes. In order to investigate defects during the transition from spermatogonia to spermatocytes, Rmb5 mutant versus wild type day 3 and 7 postnatal testes were stained for PLZF as a marker for undifferentiated spermatogonia. The number of undifferentiated spermatogonia (PLZF+ cells) per tubule observed in postnatal day 3 in the Rbm5 mutant testes was normal, however, a significant reduction compared to that in wild type animals was seen at postnatal day 7, suggesting a failure of spermatogonial commitment. Consistently, FACS analyses of the adult testes showed a significant increase in number of undifferentiated spermatogonia (PLZF+ cKit-/K67-) in the mutant compared to wild-type testes. Taken together, our findings define for the first time a critical role for RBM5 in spermatogonia differentiation.

Dynamin 2: an essential regulator of male germ cell development
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The dynamin (Dnm) family are a group of GTPases best known for their roles in membrane fission and fusion events such as endocytosis, as well as in the regulation of cytoskeletal components. A role for Dnm 1/2 has recently been identified during the acrosome reaction in spermatozoa, however little is known about Dnm function in the early male germine. We examined the expression profiles of the three mammalian Dnm genes (Dnm 1,2 and 3) in the mouse testes and isolated male germ cell/spermatocytoid, and identified Dnm1 and 2 as the only family members expressed at detectable levels in germ cells. To examine consequences of Dnm2 loss in the male germ line, we created an male germ cell-specific Dnm2 mouse knockout, (DDX4Cre;Dnm2 lox/lox). Dnm2 knockout animals failed to form mature spermatozoa with complete spermatogenic arrest at the zygote stage of meiotic development. Analysis revealed significant apoptosis in cells at post natal D15 and a complete absence of pachytestine spermatocytes in all null animals. To further examine Dnm2 function in the male germ line, we characterized the phosphorylating kinase and phosphatase to establish the molecular switch mechanisms in the meiotic and post-meiotic cells. Our studies indicate that Dnm2 is essential for male germ cell development during the early phases of spermatogenesis, and that other Dnm family members are unable to compensate in the absence. We also identified a unique role for Dnm2 in regulating meiosis, which highlights key features of the role of Dnm2 in chromosome dynamics.

Delineating the role of Snail transcription factors in the testis
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Snail transcription factors induce the epithelial-mesenchymal transition (EMT), as epithelial cells acquire migratory/invasive properties of mesenchymal cells to mediate embryonic development and tumour progression. Cells undergoing EMT exhibit stem-cell-like traits, including decreased E-cadherin expression (1), raising the question of whether genes involved in EMT also function in stem cells. In several organs, including intestine, Snail proteins are also present in multipotent cells. An initial description of Sna1-deficient mice revealed males exhibit reduced seminiferous tubule size and infertility (2), implicating Sna1 in normal spermatogenesis. However, expression and function of the three mammalian Snails (1-3) are yet to be elucidated in the mammalian testis. This study defines the cellular distribution of Snail mRNAs and proteins in mouse and human testes. In situ hybridisation of adult mouse testis revealed Sna1 and Sna2 are predominantly detected in spermatogonia and spermatocytes; Sna3 is similar, but more widespread, with signal also detected in all spermatogenic stages and in Sertoli cells. Immunohistochemistry on adult mouse testis detected Sna1 in round and elongated spermatid nuclei between Stages IX-XII, Sna2 in the cytoplasm of all germ cells, and Sna3 in the Sertoli cell cytoplasm. In the developing mouse testis, Sna1 is robustly detected in the
Spermatogenesis is an elaborately regulated system dedicated to the continuous production of spermatozoa via the genesis of spermatogonia. In this process, a variety of genes are expressed that are relevant to the differentiation of germ cells at each stage. Although Notch signaling pathway plays a critical role in germ cell development in Drosophila and Caenorhabditis elegans, its function and importance for spermatogenesis in mammals is controversial. We report that Nkapl (NFB Activating Protein-Like) is a novel germ cell-specific transcriptional suppressor in Notch signaling pathway. This protein is presumably originated from Nkap (NFB Activating Protein) due to its high homology in amino acids and conserved domains. In mice Nkapl is expressed robustly in the testis from age of 3 weeks and localized to the nuclei of spermatogonia and early spermatocytes. We have shown that Nkapl can interact with several molecules of the Notch corepressor complex, such as CiR, HDAC3, and CSL, which regulate the transcription level of the downstream genes. Furthermore, Nkapl suppressed the transcription of Notch signaling pathway through the CSL binding site, a Notch signaling specific binding site, in luciferase assay. In order to understand the function of Nkapl, we have generated Nkapl knockout mice. Male germ cells showed a complete developmental arrest at meiosis, and significant apoptosis was observed at pachytene spermatocytes. Real-time PCR revealed the significant increases of downstream genes in Notch signaling in the testes of the knockout mice, compared to wild type. Additionally, significant changes were found in differentiation markers and apoptotic factors. In summary, our data suggests that Nkapl is indispensable for male fertility, and aberrantly elevated Notch signaling has negative effects on spermatogenesis. Our results add a new dimension to the complexity of Notch signalling within the testis.

Assessment of capacitation-like changes in the spermatozoa of the Australian saltwater crocodile

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It is well established that mammalian spermatozoa acquire functional maturity during two key phases of post-testicular development. The first of these occurs as the cells are conveyed through the male reproductive tract (epididymal maturation) and the second as they ascend the female reproductive tract (capacitation). However, the degree of post-testicular development necessary to achieve fertilisation in other vertebrates remains far less clear. Indeed, despite reports that the Wolffian duct of aves and reptiles is capable of secreting proteins that bind and modify the sperm surface characteristics, it remains contentious whether capacitation is a pre-requisite for fertilisation in these species. This study was therefore undertaken with the aim of exploring whether reptile sperm do undergo capacitation-like changes following ejaculation. For this purpose we assessed the behaviour of Australian saltwater crocodile (Crocodylus porosus) spermatozoa in response to incubation under conditions that have been optimised for the induction of capacitation in mammalian species. These studies revealed that crocodile spermatozoa experienced a rapid, cyclic-AMP mediated increase in progressive motility that was accompanied by elevated levels of tyrosine phosphorylation predominantly localised within the sperm tail. These characteristic hallmarks of capacitation were also accompanied by a concomitant increase in the capacity to undergo an ionophore induced acrosome reaction. Among the key targets for tyrosine phosphorylation, we identified an orthologue of outer dense fibre 2 (ODF2), a protein that also undergoes capacitation-associated tyrosine phosphorylation in mammalian spermatozoa. Overall these data support the concept that reptilian spermatozoa do undergo capacitation-like changes in preparation for fertilisation of an ovum. Such findings are likely to find application in the development of innovative assisted reproductive strategies for captive populations of crocodiles.
Exposure to ammonium during pre-implantation embryo development significantly alters offspring phenotype.

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Adult health can be programmed by conceptus adaptations to environmental conditions during pre-implantation development. During IVF the embryo is exposed to conditions which may impact viability including temperature and toxin build-up (e.g. ammonium). Ammonium levels can increase in culture media due to deamination of amino-acids, decreasing embryo quality, implantation and fetal development, however its impact on offspring health is unknown. Therefore the aim of this study was to investigate the impact of ammonium exposure on offspring phenotype.

Zygotes from CBAF1 mice were cultured in sequential media ± 300µM ammonium. Blastocysts were transferred to recipient mothers. From d18 of pregnancy, females were housed individually and monitored for live birth. Pups were tattooed on d3 for tracking and weighed on d3 to d21. At d21, pups were sexed, weaned and weighed weekly until 17weeks when post mortems were performed.

Exposure to 300µM ammonium significantly decreased embryo viability, with reductions in viable offspring/embryo transferred (46% vs. 36%, p<0.05). Ammonium exposure increased female pre-weaning weights at d14 (6.8g±0.4 vs. 7.9g±0.4) and d21 (8.2g±0.5 vs. 9.7g±0.6, p<0.05) and this difference was maintained until 5 weeks of age. Although no change in weight was seen by 17 weeks, post mortem analysis revealed female mice had significantly increased retro-peritoneal and gonadal fat deposits and increased total adipose tissue, both in mass (0.59g±0.8 vs. 0.92g±0.9) and percentage body weight (2.6%±0.3 vs. 3.9±0.5, p<0.01). No difference was seen in male weight however male mice exposed to ammonium showed signs of insulin resistance, evidenced by a decreased AUC after ITT (p<0.05).

Exposure to ammonium throughout embryo development results in female offspring with increased adipose accumulation and male offspring with early evidence of insulin resistance. This demonstrates how the preimplantation environment influences offspring phenotype and that culture media used in IVF should contain stable forms of amino-acids to minimise ammonium build-up.

Phthalate ester-induced cytoskeletal disruption in early embryogenesis of an Australian native marine invertebrate Galeolaria gemineoa (Polychaeta: Serpulidae)

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Galeolaria gemineoa is a marine invertebrate inhabits along the mid-littoral zone of Australia’s east coast. As a broadcast-spawning species, G. gemineoa releases its gametes to the water column before fertilization, which provides more opportunity for the gametes being exposed to marine pollutants. In this study, the toxic effect of six phthalate esters (PAEs, common environmental pollutants) on the early embryogenesis was investigated by conducting sperm- and oocyte-exposure tests.

Sperm exposure to PAEs resulted in decreases of embryogenesis success in a dose-dependent manner and embryonic abnormalities with a distinctive pattern, while oocyte exposure mostly had no effect. Exceptionally, oocyte exposure to a relatively high concentration of dibutyl phthalate (20 mg/L) induced a dramatic decline in the success of embryogenesis. Based on these tests, we concluded that impaired embryonic development originates largely from PAE-induced damage to the male germ line.

Instead of undergoing a symmetrical and synchronous embryonic cleavage, the PAE-exposed spermatozoa resulted in abnormal embryos with disrupted karyokinesis and cytokinesis. From 2-cell stage, only one blastomere could divide further while the other one was arrested. Double staining for microtubules and chromosomes revealed that the mitotic spindles in the abnormal embryos were disorganised, shortened and unanchored to the cytoplasmic membrane, which resulted in unequal segregation of chromosomes. Within the non-dividing blastomeres of such embryos, nuclear divisions were found to continue as indicated by the presence of multiple spindle poles. However, cytokinesis had been disrupted due to a failure to form contractile actin rings. This abnormality indicated that in addition to any sperm DNA damage, PAEs induced changes in the sperm centriole, leading to a disruption of cytoskeletal proteins during cleavage. Such sperm damage could originate from oxidative stress caused by PAE-induced intracellular suppression of superoxide dismutase.

This study highlights the potential of this species for monitoring developmental toxicants in the marine environment.
Autocrine ligands activate a canonical immediate early gene and late gene response at the time of embryonic genomic activation in the mouse 2-cell embryo

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We have previously shown that the autocrine embryotropin, Paf, activates members of the CREB family of transcription factors at the time of embryonic genome activation. These transcription factors act on the CRE response element of the genome, which is present in over 4000 potential target genes. Included in these targets are many genes that encode other transcription factors. In this study we examined the response of a range of transcription factor genes to exogenous Paf challenge. We show that the mouse 2-cell embryo responds with a typical pattern of transient immediate early response gene (IEG) expression. This is followed by a secondary wave of late response gene expression. The IEG response was not dependent upon the synthesis of new proteins, and included the expression of cFos, cJun, Jund, Egr1, Fosl2, Sp4 and Cebpb. All of these transcription factor genes were induced within 20 - 40 min of Paf exposure and are known to be CRE-responsive. The products of cFos and cJun can dimerize to form the active AP1 transcription factor. We show that a range of AP1-responsive genes (cMyc, Klf4, Stat6, Smad3, Nanog and Nfatc1) were also induced by Paf but showed increased but delayed expression (60 - 120 min) and was dependent upon protein synthesis. Further analysis showed that Paf treatment increased cellular accumulation of immuno-detectable c-MYC and NANOG in the two-cell embryo. Together with earlier studies that showed Paf caused the activation (by phosphorylation) of pre-existing CREB and ATF1 transcription factors, these results implicate cell stimulation by autocrine ligands at the time of embryonic genome activation as a mechanism for generating a diverse new transcriptome within the embryo at the time of embryo genome activation. This occurs via canonical immediate and late gene expression responses that are characteristic of trophic stimulation of somatic cells.
Oxidative Stress-Induced Protein Modifications in Spermatozoa and Consequences for Sperm-Oocyte Recognition

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Male infertility is a distressingly common condition affecting at least 1 in 20 men. In recent years, oxidative stress has been shown to be exceedingly common in the infertile male cohort. We have observed that reactive oxygen species plays a key role in sperm dysfunction and indeed products of oxidative stress, nucleophilic aldehydes, may adduct and disrupt the molecular chaperone HSPA2. This protein is responsible for facilitating the expression of an oocyte-receptor complex to the sperm surface, essential for processes leading to fertilisation. Recent data has implicated two client proteins, SPA and ARSA that form a zona pellucida–receptor complex regulated by the molecular chaperone HSPA2. We have used a combination of molecular, mass spectroscopy and molecular modelling approaches to study the structure of the oocyte-receptor complex and further, to identify the molecular mechanisms arising from oxidative stress that lead to disruption of complex formation and therefore failure to bind to the oocyte.

Our preliminary data has revealed that under a state of oxidative stress, HSPA2 becomes alkylated by reactive aldehydes, specifically 4-hydroxynonenal (4HNE). We have also identified adduct formation at Cys267, an amino acid that is critical for the chaperoning activity of HPSA2. This is consistent with the demonstration that 4HNE adduction disrupts HSPA2-client protein interactions (assessed by blue native), leading to dissociation of HSPA2 complexes. We are now focused on detailing the molecular nature of HSPA2 modifications driven by oxidative stress. We also aim to understand the structural impact of this damage and how it specifically influences the ability of HSPA2 to orchestrate oocyte-receptor complex assembly.

As oxidative stress is an emerging aetiology for male infertility, understanding the fundamental mechanisms behind the loss of sperm-oocyte binding, provides a strong platform for the rational design of targeted antioxidant therapies, tailored to this specific class of male infertility.

Dietary micronutrient supplementation to a high fat diet reduces sperm oxidative stress and improves fertilisation rates in a mouse model

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Male obesity is associated with impaired reproductive health. One potential mechanism common to multiple causes of subfertility is oxidative stress in sperm. Dietary supplementation with antioxidants reduces sperm oxidative stress in normal weight men. However, the effects of dietary micronutrient supplementation in obese males on sperm quality and function have not been investigated to date.

We used a mouse model of diet induced obesity (i.e. a high fat diet; HFD) to examine the effects of dietary micronutrient supplements with anti-oxidative properties on sperm quality and function for a duration that spans two rounds of spermatogenesis (10 weeks). Sperm measures of motility, concentration, morphology, intracellular reactive oxygen species (ROS), oxidative DNA damage, capacitation, binding and fertilisation rates were examined. In addition, body composition, glucose and insulin tolerance; serum metabolites, testosterone and C-Reactive Protein (CRP) concentrations were also examined.

Mice fed a HFD supplemented with micronutrients had reduced total adipose tissue mass with no changes in serum metabolites, testosterone, CRP or glucose and insulin tolerance compared to mice fed a non-fortified HFD. Micronutrient supplementation of a HFD improved normal sperm morphology, reduced sperm intracellular ROS and reduced 8-hydroxyguanosine (8-OHdG) fluorescence, a biomarker for oxidative DNA damage, with no effect on sperm motility or concentration. Furthermore, sperm capacitation, binding and fertilisation were also improved when mice consumed a HFD supplemented with micronutrients, which are normally compromised by the HFD regime.

This study demonstrates that impaired sperm quality and function resulting from male diet induced obesity can be improved by micronutrient supplementation for 10 weeks, without a change of diet. Moreover, given that obesity is often linked with a lack of basic nutrients including essential vitamins and minerals, our findings suggest that micronutrient supplementation may attenuate obesity associated nutrient deficiencies ultimately improving spermatogenesis and sperm quality and function.

The vitamin D receptor (VDR) is expressed in skeletal muscle of male mice and modulates 25-hydroxyvitamin D (25OHD) uptake in myofibers

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For centuries, children with rickets were reported to demonstrate muscle wasting, weakness and hypotonia. Similarly, adults with vitamin D deficiency commonly display myalgia, muscle weakness and type 2 muscle fibre atrophy, which reverse with vitamin D supplementation. However, vitamin D’s effects in muscle are elusive. Whether its effects in muscle are predominantly...
indirect, via calcium and/or phosphate levels, or direct has been debated for decades. The central question is whether the vitamin D receptor (VDR) is expressed in skeletal muscle and directly alters muscle physiology. In this talk, research evaluating the VDR in muscle, experimental models assessing vitamin D signalling in this tissue and technical reasons for the controversial nature of this field will be presented.

Des-acyl ghrelin inhibits oestrogen-stimulated breast tumour growth in vitro and in vivo

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Background: The majority of breast cancers are oestrogen receptor positive (ER+). The aromatase enzyme catalyses the conversion of androgens into oestrogens and its expression in breast adipose is a major driver of oestrogen-dependent breast cancer after menopause. Aromatase inhibitors are currently first-line therapy for ER+ breast cancer, but their use is also associated with side-effects due to inhibition of aromatase in bone. Our lab has discovered that the gut-derived peptide hormone des-acyl ghrelin (DAG) inhibits the growth of ER+ breast cancer cells as well as aromatase activity.

Aims and hypotheses: We hypothesise that DAG may be efficacious for the treatment of ER+ breast cancer. We aim to examine the effect of DAG on aromatase activity and breast tumour growth in vitro and in vivo.

Methods: Effect of DAG on the oestrogen-dependent proliferation of breast cancer cell lines (MCF7, ZR75) was examined by quantifying EdU incorporation in vitro and in vivo. In vitro studies were performed using 3D cultures, whereas the effect of DAG in vivo was examined in mammary fat pad-xenografted balb/c nude mice. The effect of DAG on aromatase activity was examined in breast cancer explants using the titrated water-release assay.

Results: DAG (10pM, 100pM and 1000pM) significantly inhibits the oestrogen-stimulated number and proliferation of MCF7 and ZR75 cells in vitro (n=3; P≤0.05). Consistently, DAG (10ug/kg and 100ug/kg) also significantly inhibits MCF7 and ZR75 (n=3/group; P≤0.0001) tumour growth in the presence of oestradiol in vivo compared to vehicle control. Moreover, DAG inhibits aromatase activity at 10pM and 100pM (P≤0.005) in breast cancer tissue explants.

Conclusions: Our findings suggest that DAG will inhibit breast cancer growth via direct effects on cell proliferation and indirect effects on oestrogen production. Therefore, DAG or DAG mimetics may be useful as possible ER+ breast cancer therapeutics in the future.

Combined PPARγ and XIAP treatment sensitises granulosa cell tumours to PPARγ-mediated apoptosis

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Ovarian granulosa cell tumours (GCT) are hormonally active cancers characterised by indolent growth and late, invasive relapse. Aside from surgery, the therapeutic options are very limited. We previously reported (ESA 2014) the nuclear receptor, peroxisome proliferator-activated receptor-gamma (PPARγ), and the X-linked inhibitor of apoptosis protein (XIAP) to be potential specific therapeutic targets for the treatment of GCT. Inhibiting XIAP releases PPARγ transrepression by the constitutive NF-κB activity, and together with PPARγ activation, induces apoptosis in two GCT-derived cell lines, KGN and COV434. Our aims are to: 1) identify differentially expressed proteins after combined PPARg and XIAP treatment; and 2) investigate the mechanism by which XIAP inhibition abrogated NF-κB transrepression of PPARγ in GCT-derived cells.

We have used stable isotope labelling with amino acids in cell culture (SILAC), a proteomic approach to identify differential expressed proteins after combined PPARg activation (rosiglitazone) and XIAP inhibition (Smac mimic) for 24 hours. The role of upstream regulator of NF-κB, TGF-β-activated kinase (TAK) 1, which is activated via XIAP, was investigated using transactivation assays and a specific TAK1 inhibitor, 5Z-7-oxozeaenol.

In KGN and COV434, we identified a total of 407 proteins, 31 of which were upregulated by ≥1.5 fold with the combined treatment. These included several proteins involved in metabolic processes such as acyl-CoA desaturase (4.50-fold), phosphoglycerate kinase 1 (2.87-fold) and α-enolase (1.75-fold). We demonstrated that the inhibition of XIAP and TAK1 significantly reduced NF-κB-mediated transactivation in GCT in vitro.

As PPARγ plays a pivotal role in lipid and glucose metabolism, upregulation of proteins associated with metabolic processes is consistent with the restoration of PPARγ activity. Constitutive NF-κB signalling is potentially a consequence of a positive feed-forward loop involving its effector protein XIAP. Findings suggested that TAK1 may also be involved in constitutive NF-κB signalling in GCT in vitro.
Comparative Effects of Endogenous and Synthetic Glucocorticoid Steroids during Mammalian Lung Development

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Preterm babies are born with a high risk of respiratory distress syndrome (RDS), as their lungs are often too immature to efficiently respire without the assistance of mechanical ventilation. This is partly due to preterm babies being born before the preparturient fetal glucocorticoid surge, which matures the lung in preparation for birth. Currently, the only treatment for lung immaturity in preterm babies is maternal administration of synthetic glucocorticoids. However there are some adverse side effects associated with synthetic glucocorticoid use, such as a decrease in overall fetal growth and delayed brain development, which are not observed with endogenous glucocorticoids. Despite their routine use, the genomic mechanisms surrounding glucocorticoid-mediated lung development remain poorly characterised. We propose that synthetic and endogenous glucocorticoids differentially regulate specific, but different, subsets of genes leading to rapid lung maturation in the preterm, but also the inadvertent modulation of off-target genes that are possibly linked to various adverse side effects. To identify these specific gene sets, fetal rat lung fibroblast cells, isolated from Sprague Dawley rats at E20 (term ~E22), were treated for a period of 6 hours with either synthetic (Betametasone or Dexamethasone 10⁻⁶M), endogenous glucocorticoids (Corticosterone 10⁻⁶M) or vehicle as a control (0.01%). Using Next Generation RNA-sequencing (RNA-seq) we found that the overall gene expression profile is similar for both endogenous and synthetic glucocorticoids. However synthetic glucocorticoids modulated most of these genes to a higher degree compared to endogenous glucocorticoids. Quantitative PCR of novel lung specific genes modulated by glucocorticoids include Nephroblastoma overexpressed (NOV) and Transglutaminase 2 (Tgm2) showed a significantly higher expression (p<0.05) in lung fibroblast cells treated with betamethasone or corticosterone compared to fibroblasts treated with vehicle. By gaining a better understanding of the mechanisms driving glucocorticoid mediated lung development it will be possible to development better lung-specific treatments for preterm babies born with RDS, as their lungs are often too immature to efficiently respire without the assistance of mechanical ventilation.

The refinement of Luteinizing Hormone (LH) output during pubertal maturation is associated with the recruitment of follicle in adult female mice

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Granulosa cell-specific or global Pten mutations in combination with transgenic FSH expression fails to induce ovarian tumors.

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PTEN mutations occur frequently in ovarian cancer but in previous experimental studies granulosa cell (GC)-specific Pten inactivation alone was insufficient to cause tumour formation. Follicle stimulating hormone (FSH) is vital for ovarian function with elevated circulating levels associated with reproductive ageing and ovarian tumorigenesis in women. Transgenic FSH (TgFSH) mice exhibit progressively rising FSH levels with ageing causing ovarian dysfunction and premature infertility, but no tumours. We hypothesized that high FSH when combined with ovarian Pten mutations may promote ovarian tumorigenesis in mice.

We used Tg.AMHH.Cre to target Pten disruption to GCs (Pten−/−) and Tg.Sox2.Cre for global heterozygous Pten mutation (Pten+/−) combining them with TgFSH overexpression as a multi-hit strategy to use genetic and hormonal means to induce ovarian tumors. TgFSH ± Pten−/− females displayed similar ovary and uterine weights versus controls (±TgFSH) with no detectable tumours at 12 months. TgFSH ± Pten−/+ ovary weights remained unchanged (p = 0.5), whereas uterine weights increased due to tumors vs controls (p < 0.001). TgFSH ± Pten−/+ females developed tumours in various organs (pituitary, skin, kidneys) but not in the ovary. Estrous cycling and stage lengths were not significantly altered by ovary-specific Pten−/+ status whereas global Pten−/+ mice had reduced estrous cycling (p < 0.001). Corpora lutea CL numbers remained unchanged among Pten−/+ mice vs control (p = 0.99), however Pten−/− significantly increased CL numbers (p < 0.05), indicating increased ovulation rates or persisting CLs by global Pten mutation.

We conclude that specific follicular or global Pten mutations, alone, or combined with TgFSH, were not sufficient to cause ovarian tumors. These findings that the ovary is remarkably resistant to oncogenesis support the newer extra-gonadal origin hypothesis for ovarian tumorigenesis.

Major histocompatibility complex class I genes at the fetal-maternal interface of a marsupial (Macropus eugenii)

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Major Histocompatibility Complex class I molecules (MHC-I) are expressed at the cell surface and are responsible for the presentation of self and non-self antigen repertoire to the immune system. Eutherian mammals including mice and humans express both classical and non-classical MHC-I molecules in the placenta, the latter of which are thought to modulate the maternal immune response during pregnancy. Marsupials last shared a common ancestor with eutherian mammals such as humans and mice over 160 million years ago. Since, like eutherians, they have an intra-uterine development dependent on a placenta, albeit a short-lived and less invasive one, they provide an opportunity to investigate the evolution of MHC-I expression at the fetal-maternal interface. We have characterised MHC-I and β2-microglobulin (β2m) mRNA expression in reproductive tissues of the tammar wallaby (Macropus eugenii) from the time of placental attachment to day 25 of the 26.5 day pregnancy. For placental samples we sequenced 10 PCR clones amplified from universal MHC-I primers at days 18, 19, 21, 24 and 25 of pregnancy. Putative classical MHC-I genes and β2m were expressed in the chorio-vitelline placenta, fetus and gravid endometrium throughout the whole of this period. MHC-I classical sequences isolated from placenta were phylogenetically most similar to the Maae-UC (50/100 clones) and Maae-UA genes (7/100 clones). Maae-UA and Maae-UC were also the most highly expressed MHC-I genes in tammar placental transcriptome data. However, expression of these classical MHC-I genes was much higher in fetal tissue compared to the placenta suggesting differential expression between these tissue types. Expression of three non-classical MHC-I genes (Maae-Ud, Maae-Uk and Maae-Um) was also present in placental samples. The results suggest that expression of classical and non-classical MHC-I genes in extant marsupial and eutherian mammals may have been necessary for the evolution of the ancestral therian placenta and survival of the mammalian fetus.

Toll-like receptor 4 antagonist (+)-naloxone prevents infection-driven fetal loss and preterm labour in mice

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Preterm delivery (PTD) can effect up to 13% of pregnancies in developed countries with as many as half of these caused by infection-driven inflammation. Engagement of the bacterial component lipopolysaccharide (LPS) with toll-like receptor 4 (TLR4) is a well-established trigger of PTD and poor neonatal outcomes in experimental mouse models. We aimed to investigate whether (+)-naloxone, a small molecule TLR4 antagonist, can prevent the deleterious effects of LPS on pregnancy in a mouse model. Pregnant mice were challenged against either LPS (i.p.) or heat-killed E. coli (10^8 CFU) in late gestation, both of which induced PTD and fetal loss. Co-administration of (+)-naloxone (the non-opioid isomer of naloxone) acted to reverse the effects of LPS and E. coli. Gene expression analysis revealed that mice given E. coli had a surge in pro-inflammatory cytokines, including Il1a, Il1b, Il6 and Tnf in the uterus, decidua, placenta and fetal membrane, while treatment of infected mice with (+)-naloxone curbed cytokine expression. To examine the effect of LPS and (+)-naloxone on the health of surviving progeny, pups were weighed fortnightly until 20 weeks of age, with body composition determined by dual energy x-ray absorptiometry (DEXA) scans at 8, 14 and 20 weeks of age and a 20 week autopsy. Female offspring were largely unaffected, however male offspring exposed to LPS in utero had less muscle mass and more fat mass, as measured by DEXA and autopsy, compared to offspring exposed to vehicle. Administration of (+)-naloxone corrected the LPS induced body composition change in the male progeny. Collectively these data demonstrate that TLR4 ligation by infectious agents plays an important role in initiating the pro-inflammatory response which leads to PTD and fetal loss, and that (+)-naloxone is worthy of consideration as a candidate therapeutic agent for women at risk of PTD.

**Novel non-competitive Interleukin-1 receptor antagonist prevents LPS-induced preterm birth**

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Premature birth is a common and critical health issue in fetal-maternal medicine with long-term consequences especially for early preterm neonates. The pathobiology is poorly understood and the causal factors uncertain, but inflammatory mechanisms are clearly implicated. Infection-associated preterm birth (PTB) is triggered when bacterial products including lipopolysaccharide (LPS) bind Toll-like receptors (TLRs) to activate inflammation. This results in premature induction of uterine activation proteins to induce myometrial contractions and PTB in mice. Pro-inflammatory cytokine interleukin-1 beta (IL1b) has been identified as a major upstream agent, immediately proximal to TLR activation, in the inflammatory pathway to PTB. This project seeks to investigate (1) whether inhibition of IL-1 signalling using a small peptide non-competitive IL-1 receptor (IL-1R) antagonist rytena may prevent the parturition cascade caused by LPS-induced inflammation and (2) the consequences of in utero exposure to IL-1R antagonist for perinatal outcomes and post-natal development of the resulting progeny. Pregnant B6 females were treated with LPS or PBS, with or without co-administration of IL-1R antagonist, on gestational day 16.5 and allowed to deliver pups (n=10-12/group). LPS-induced PTB was successfully alleviated using IL-1R antagonist in B6 mice, preventing fetal loss associated with death in utero and/or early delivery. IL-1R antagonist resulted in on time birth with normal perinatal characteristics and pup survival rates. Litter sizes, birth weights, survival to weaning and weight at 3 weeks of age were significantly increased compared to mice administered LPS alone (P<0.05), and not different to perinatal outcomes for control, carrier-treated mice. Early intervention with IL-1R antagonists to suppress the downstream inflammatory cascade can inhibit the progression of LPS-induced PTB by preventing the premature activation of uterine activation proteins and subsequent onset of labour in mice. The IL-1 pathway warrants further investigation as a potential target for new prevention or treatment options in women with infection-associated preterm delivery.

**Delineation of myeloid lineage derived immune cells in the adult mouse testis**

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The ability of the testis to tolerate spermatogenic cell autoantigens is crucial for sperm production and any compromise can lead to male infertility. Although the testicular immune cells in the adult have been studied in detail [1-3], the developmental dynamics of these populations have yet to be characterised. Macrophages are commonly studied in inflammatory diseases; their role in organogenesis is an emerging topic, particularly relating to testis. In adult testes, macrophages closely surround Leydig cells, suggesting they communicate directly [1]. Ethane dimethane sulphonate-induced loss of Leydig cells caused macrophage depletion, and macrophage recovery occurred only after Leydig cell repopulation, highlighting the interdependence of these two cell types [1]. Macrophages have also recently been implicated in fetal testis cord morphogenesis and vascularization [2]. We hypothesised that postnatal tests development is also associated with significant changes in myeloid-derived leukocytes. To address this, we are using immunofluorescence to visualise the localisation of testicular...
macrophages and dendritic cells within adult testes of transgenic CXCR1-GFP (macrophage) and CD11c-YFP (dendritic cell) mouse models. In addition, flow cytometry experiments will quantify these cells using interstitial cells collected from mechanically dissociated adult mouse testes. Antibodies [CD45 (leukocytes), F4/80 (macrophages) and CD11c (dendritic cells)] were used to identify immune cell populations. Matched isotype controls in a fluorescence minus one setup and single stain controls enabled optimization for flow cytometry parameters and post-analysis gating of leukocytes. Samples were analysed using a BD LSR II flow cytometer. Approximately 2.5-3% of testicular cells isolated were leukocytes with macrophages accounting for the majority (2.2-2.5% of total). Furthermore, a unique population co-expressing CD11c+F4/80+ was identified for the first time in the testes. A combination of immunofluorescence and flow cytometry studies using transgenic mouse models will reveal the dynamic composition of testicular immune cells and their possible contribution to organ development and maintenance.


Expression of the Stem Cell Marker mTert Identifies Epithelial, Endothelial and Leukocyte populations in the Mouse Endometrium.

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Stem/progenitor cells are thought to be responsible for the regenerative capacity of the endometrium. However the role of these cells has been difficult to investigate in mouse models due to the lack of a marker for easy identification and tracking. Mouse telomerase reverse transcriptase (mTert) is the rate limiting component of the telomerase complex that stem cells use to escape senescence, and a stem cell marker in bone marrow and intestine. We have used transgenic mice with reporter constructs for mTert to identify and characterize putative endometrial stem/progenitor cells. Endometrial mTert expression was examined by microscopy and flow cytometry in mice expressing GFP under the control of the mTert promoter (mTert-GFP). The fate of endometrial mTert cells was examined in mice featuring inducible permanent mTert-Cre-mediated expression of tdTomato in the mTert lineage (mTert-CreER::R26R-tdTomato). CD45 leukocytes were the most abundant mTert-GFP cell type and are likely to account for the majority of telomerase activity in the endometrium. The abundance of mTert-GFP leukocytes was decreased by ovariectomy, indicating that their recruitment/maintenance is promoted by ovarian hormones. mTert-GFP leukocytes are likely to be a transient bone marrow-derived population rather than endometrial stem/progenitor cells. Rare populations of intrinsic (CD45<sup>−</sup>) mTert-GFP were detected in the endometrium and , unlike CD45<sup>+</sup> leukocytes, the relative percentage of CD45<sup>−</sup> mTert-GFP cells did not decrease in ovariectomised mice, indicating their presence is independent of ovarian hormones. This is in keeping with the concept of endometrial progenitor populations that survive hormonal deprivation. The CD45<sup>−</sup> mTert-GFP population included cells expressing epithelial (EpCAM) and endothelial (CD31, von Willebrand factor) markers. Lineage tracing using the mTert-CreER::R26R-tdTomato mouse showed mTert expressing cells contribute to epithelial and leukocyte lineages in the endometrium. Our results provide evidence that mTert-expressing cells are involved in replenishing and regenerating epithelial and endothelial structures of the endometrium.

Sex specific effects of early gestational diet upon the developing immune system

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This study investigates the effect of early gestational diet on the vulnerability of the male fetus to perturbation of the developing immune system. A sex specific response to infectious disease in neonates has been reported with females displaying a more robust cell mediated and humoral response[1]. In a unique series of experiments we are the first to show that the male foetal calf has enhanced susceptibility to early gestational diet as it shows significantly greater vulnerability; affecting male fetal growth (p=0.01) placenta (p=0.04) and importantly immune system development; including thymus growth (p=0.04), antimicrobial use in neonate (p=0.05) and protective immunity for clostridial diseases assessed via IFNγ+ and IL-13. These findings support our previous studies [2] that show male and female foetuses institute different mechanisms via the placenta in response to altered environment in the development of the immune system.
Bos indicus cross heifers (n=350) were individually fed high (14%) or low (7%) crude protein (CP) from 60 days prior to conception. At 23 days post-conception the two groups were further split into high or low % CP creating four treatment groups: High/High (HH), Low/High (LH), High/Low (HL) and Low/Low (LL).

Fetal size was reduced by the low protein diet with this effect being greater in the male at 36dpc (p=0.001). At 98dpc fetal measures and organ development were measured (n=48). Low protein decreased male thymus and placental size (p=0.04). Low first trimester protein increased neonatal antimicrobial use. At 10mth of age (post clostridial vaccination) protective immunity assessed via IFNγ- and IL-13 assessment showed periconception low protein reduced (p=0.05) IFNγ- in male calves.

Conclusion: Protein supplementation during the pre breeding and periconception period in range heifers may increase immune function in the neonate and decrease susceptibility to contagious disease.


High FSH levels alter oocyte in vitro maturation but not oocyte aneuploidy in a transgenic mouse model.

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Follicle stimulating hormone (FSH) is vital for ovarian function and serum FSH increases with age as ovarian function declines towards menopause. We hypothesize that elevated FSH may rescue follicles from a diminishing pool normally excluded from selection and thereby reducing oocyte function. Transgenic FSH (TgFSH) mice expressing progressively rising FSH levels with age displayed increased litter size initially but after 6 months of age caused decreased litter size and premature infertility due to increased embryo-fetal resorption; however, the specific mechanism was undefined. We hypothesized that increased circulating FSH exceeding a threshold impaired oocyte development and functionality.

We examined oocyte in vitro maturation and aneuploidy (CREST immunofluorescent staining) and cumulus cells (from cumulus-oocyte complexes) for gene expression analysis (qPCR) in TgFSH and non-TgFSH control mice aged 6, 12, 18 and 24 months. Oocytes of TgFSH mice exhibited increased accumulation in the GV stage (20%, p < 0.05) accompanied by reduction in MII stage of maturation (10%, p < 0.05) vs age-matched littermate controls. The reduced oocyte progression to the MII stage is attributable to stalling of the oocytes in the GC stage. The proportion of aneuploid oocytes increased with age (p < 0.001) but did not differ between genotypes (p = 0.83).

We conclude that high FSH has detrimental effects on oocyte maturation/development but does not increase the aneuploidy rate. This suggests that the previously observed age-related subfertility of TgFSH female mice older than 6 months of age is not due to aneuploidy but could be due to other aspects of oocyte health/functionality and warrants further investigation.

SIRT2 over-expression reverses ageing-induced decline in oocyte quality

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Oocyte quality deteriorates markedly with ageing leading to disordered spindle assembly and reduced defense against deleterious reactive oxygen species (ROS). Sirtuins are a family of seven (SIRT1-7) NAD+-dependent deacetylases with potent anti-aging properties. We recently showed that in somatic cells, SIRT2 stabilizes Budding uninhibited by benzimidazole-Related 1 (BubR1), which we previously showed controls oocyte spindle assembly and declines with ageing in oocytes. Here we studied oocytes from a transgenic mouse that overexpresses SIRT2 (SIRT2-Tg mice). BubR1 was increased in SIRT2-Tg oocytes pointing to SIRT2-dependent BubR1 stabilization as in somatic cells. To determine whether SIRT2 overexpression would impact oocyte quality during natural ageing, we aged mice to 12 months when oocyte quality is known to be poor. We first examined in vitro meiotic maturation, which commences with germinal vesicle breakdown (GVBD) and concludes with first polar body extrusion (PBE). Interestingly, by 16 h post-GVBD, almost twice as many aged SIRT2-Tg oocytes underwent PBE compared with WT (69% versus 36%; P=0.04), comparable to PBE rates observed in younger (4-month-old) WT oocytes (62%). Thus, SIRT2 overexpression reverses poor meiotic competence brought on by ageing. Strikingly, aged SIRT2-Tg oocytes also exhibited higher rates of normal spindles with aligned chromosomes both in meiosis I (70% versus 15%) and at metaphase II-arrest (44% versus 25%). Finally, following hydrogen-peroxide-induced oxidative stress, ROS levels in SIRT2-Tg oocytes were less than half that in WT oocytes (P=0.0001) indicating that SIRT2 overexpression potentiates anti-oxidant defenses. Collectively, these data show that SIRT2 combats age-induced deterioration in oocyte quality. Two possible
mechanisms could involve increased BubR1 stability and enhanced capacity for ROS detoxification. Approaches for increasing SIRT2 activity could therefore provide an attractive avenue for modulating oocyte quality.

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Permeability Glycoprotein Enhances Cellular Drug Exclusion in the Early Embryo; Upholding DNA Integrity

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At the time of fertilisation, oocyte activation initiates the upregulation of DNA repair pathways and cellular protective mechanisms to ensure that the fertilised cell is equipped to cope with the challenges of embryogenesis and later development. However, double strand breaks (DSB) within the oocyte DNA have been shown to interfere with the ability of the female gamete to generate a viable embryo, and are directly correlated with preimplantation errors, spontaneous abortion, and developmental abnormalities. The aim of this research was to characterise the intracellular mechanisms that protect oocytes against DSB-inducing molecules during the pre- (metaphase II, MII) and post-fertilisation (zygote and oocyte from parthenotes) phases of development. Immunocytochemical analyses confirmed that the common genotoxic agent etoposide, was capable of inducing potent DNA DSB damage in mouse MII oocytes (p<0.0001). Interestingly, however, the sensitivity of oocytes to etoposide was significantly decreased following fertilisation/spontaneous activation (p<0.01); highlighting substantial differences in DNA damage sensitivity in maturing oocytes. This reduced sensitivity appeared to be attributed, at least in part, to an upregulation of multidrug resistant (MDR) efflux transport activity following oocyte activation/fertilisation. In this context, we demonstrated a rapid increase in the expression of the MDR, permeability glycoprotein (PGP), and its translocation to the oolemma following oocyte activation/fertilisation. The enhanced activity of PGP in activated/fertilised oocytes was demonstrated through dye exclusion assays in the presence and absence of the selective PGP inhibitor, PSC833. These data confirmed that PGP afforded the activated/fertilised oocytes protection against etoposide, with its inhibition resulting in the induction of DNA damage sensitivity between pre- and post-fertilisation oocytes. Ongoing work will focus on assessment of the specific role of PGP and other MDR transporters in protecting the fertilised oocyte from DNA damage.

The effect of radiofrequency-electromagnetic radiation on the male germ line

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Over the past 15 years the use of mobile phones has increased considerably, with a current estimate of more than one billion users worldwide. The Australian population represents some of the most active users, recognised by the fact that on average 4 devices are in operation among every 3 people. While the impact of these devices on human health is under active debate, the electromagnetic radiation (EMR) emitted by mobile phones has been highlighted as a possible carcinogen by the International Agency for Research on Cancer. Owing to the practice of storing mobile phone devices in the pant pocket, in line with the reproductive system, it is becoming increasingly important to assess the effect of radiofrequency-EMR (RF-EMR) on the male germ line particularly as several studies have identified a potential impact of RF-EMR on sperm function and DNA integrity. In this study, mouse germ cell lines (GC1, spermatogonial like; GC2, spermatocyte like) and cauda epididymal spermatozoa were exposed to RF-EMR generated by a waveguide for 0-6 hours. Radiation mimicking that emitted by mobile phone devices (0.15 W/kg, 1.8 GHz) induced significant increases in mitochondrial reactive oxygen species production in both GC1 (p<0.01) and GC2 (p<0.05) cell lines, after 2 and 4 hours of exposure, respectively. A similar effect was not observed in mature spermatozoa, however the motility of these cells proved particularly sensitive with significant reductions (p<0.05) recorded across the categories of rapid, progressive and total motility after 4 hours of exposure. This treatment also led to a marked suppression of sperm maturation as judged by the failure of these cells to undergo capacitation-associated increases in phosphorylserine expression within their tail. Our continuing research will focus on the downstream effects of RF-EMR on sperm fertilising ability and determining the precise mechanism(s) underpinning the differential effects observed in developing germ cells.

Electrophilic aldehydes increase free radical production and modify surface proteins in horse spermatozoa

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Spermatozoa are highly sensitive to oxidative stress owing to a lack of antioxidant defences and a high proportion of polyunsaturated fatty acids within their plasma membrane. A key hallmark of oxidative stress is the production of highly reactive electrophilic aldehydes, generated as a consequence of lipid peroxidation. In this study we have assessed the impact of three
such aldehydes, acrolein (ACR), 4-hydroxynonenal (4HNE) and malondialdehyde (MDA) displaying different levels of electrophilicity (ACR>4HNE>MDA), on equine spermatozoa, a cell type that generates substantial concentrations of reactive oxygen species (ROS) as a by-product of their normal metabolism. Our study revealed that all three aldehydes readily adducted to sperm surface proteins located predominantly within the post-acrosomal region of the head, proximal centriole and tail. The impact of such adduction was manifest in a significant dose- and time-dependent decrease in sperm motility. Indeed, sperm motility was completely lost after only 3 hours of exposure to both ACR and HNE (P<0.0001). In the case of the less reactive MDA, the decline in motility was less pronounced but still proved to be significant (P<0.01) after 24 hours of treatment. Similarly, both mitochondrial and cytosolic levels of ROS were also significantly elevated following 3 hours of treatment for ACR and 4HNE (P<0.0001) and 24 hours for ACR and MDA (P<0.01). Future work will focus on refining our analysis of the functional implications of reactive aldehydes and oxidative stress on stallion spermatozoa and determining whether this information can be exploited for the development of diagnostic markers of stallion fertility and/or novel contraceptive strategies to tackle the sensitive issue of feral horse control.

Next generation sequence analysis of miRNA signatures in mouse epididymal epithelial cells and spermatozoa.

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Spermatozoa acquire functional competence as they descend through the epididymis, a highly specialised region of the male reproductive tract. A characteristic feature of the epididymis is its ability to create highly regionalised microenvironments that promote sperm maturation. Although the molecular mechanisms through which these dynamic microenvironments are generated remains to be fully elucidated, recent studies have implicated the RNA interference pathway as a key regulator of epididymal gene expression profiles. Hence, in this study we have applied next generation sequencing technology to explore the microRNA (miRNA) signature of mouse epididymal epithelial cells and that of spermatozoa maturing within the lumen of the duct. In doing so we have demonstrated substantial segmental differences in the expression of epithelial miRNAs, as well as providing the first evidence for the post-testicular modification of miRNA profiles in spermatozoa under normal physiological conditions. In total, 370 miRNAs were identified in whole epididymal tissue, 218 of which proved to be unique to epithelial cells, including a substantial portion that putatively regulate canonical signalling pathways involved in maintenance of cellular development, proliferation and cell death. Similarly, an impressive profile of some 295 miRNAs were identified in mouse spermatozoa as they enter the epididymis. This profile was however, dynamically altered following the apparent loss of 113 and acquisition of a further 115 miRNAs between the proximal (caput) and terminal (cauda) regions of the duct. Bioinformatic analyses revealed that the miRNAs present in mature spermatozoa mapped predominantly to NF-κB and TGFβ signalling pathways that have been implicated in conditioning of the peri-conceptual environment within the female reproductive tract and in regulating embryonic development. Collectively, the data presented herein provide the first evidence in support of a key role for miRNA regulation of epididymal sperm maturation in the mouse model.

The Battle of the Sexes: Establishing male or female fate of the bipotential gonad

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The gonad is an outstanding experimental model where a primary fate decision in bipotential precursor cells has the dramatic consequence of controlling the phenotypic sex of the organism. We have focused on how this fate decision is executed and maintained at the level of the transcriptional network, as well as how divergent organogenesis of a testis or ovary results from this primary fate decision. Transcripome analysis of the three principle precursor populations in the bipotential XX and XY gonad (supporting cell precursors, steroidogenic cell precursors, and germ cells) identified distinct signatures specific to each of these lineages, that are shared in XX and XY gonads. At the bipotential stage, more genes associated with the female pathway are expressed in both XX and XY supporting cell precursors, suggesting a female bias in lineage priming. In mammals, divergence along the male pathway is initiated by transcriptional activation of the Y-linked gene, Sry, at E10.5 in supporting cell precursors. To define the dynamic establishment of sexually dimorphic expression patterns downstream of Sry, we conducted transcriptome analyses comparing XX and XY gonads at six 4-hour intervals between E11.0-E12.0. Expression of Sry initiates sequential upregulation of male pathway genes, as well as sequential downregulation of female pathway genes in XY gonads. Deletion of Fgf9, which acts as a repressor of Wnt4, a signal that drives the female pathway, leads to male to female sex reversal and ovary development in XY offspring. This and other experiments suggest that repression of female genes is critical to establish the male pathway. To investigate how this fate commitment is reflected in the chromatin landscape in pre-Sertoli cells, we identified DNase hypersensitivity sites (DHS) in chromatin of E13.5 and E15.5 Sertoli progenitors. DHS identified regions of open chromatin both in genes associated with the male pathway and actively transcribed in Sertoli progenitors, and those that were silent and associated with the female pathway. Chromatin immunoprecipitation (ChIP) analysis using an antibody against the active chromatin mark, H3K27ac, distinguished those DHS peaks that are associated with active transcription from those that are likely associated with factors that repress expression, identifying enhancers across the genome, and defining the chromatin landscape that regulates commitment to Sertoli fate. This study was funded by grants from NICHD and NIDDK to BC.
Bisphosphonates and cancer: seeing old drugs in a new light

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Bisphosphonate drugs (BPs) rapidly target the skeleton and are the gold standard treatment to inhibit bone destruction in patients with osteoporosis and metastatic bone disease. However, BPs also have anti-cancer effects outside bone; in mouse models they reduce tumour growth and metastasis and there is some evidence that adjuvant BP therapy increases survival in post-menopausal women with breast cancer. The exact mechanisms underlying these anti-cancer effects are unknown since BPs are considered to only affect bone-resident osteoclasts in vivo. To address this, we determined the cell types capable of internalising fluorescently-labelled BP in mice bearing 4T1 mammary tumours. Within minutes of tail vein injection, intravital 2-photon imaging revealed the diffusion of BP into tumour tissue from the leaky, disorganised tumour vasculature. BP then appeared to bind to small, granular microcalcifications within the tumour tissue. Intravital imaging revealed that tumour-associated macrophages (TAMS) rapidly internalised BP by pinocytosis and by engulfing these BP-coated microcalcifications. Flow cytometric analysis of the tumours 24hr later confirmed that uptake occurred predominantly in TAMS and not tumour epithelial cells.

We also identified a patient with breast cancer in which the BP 99m-Tc-MDP (used for SPECT/CT bone scintigraphy) localised to the primary mammary carcinoma. Histological analysis of the resected tumour post-surgery revealed the presence of granular microcalcifications similar in appearance to those in the mouse 4T1 tumours, and some of which were closely associated with CD68+ TAMs.

These studies provide clear evidence that BPs can be rapidly internalised by macrophages outside the skeleton. Their leaky vasculature of tumours facilitates the local diffusion of BP, where it binds to microcalcifications within the tumour that are engulfed by TAMS. Given the important role of TAMS in promoting tumour progression and metastasis, our studies suggest that the anti-tumour activity of BPs in cancer patients occurs indirectly via effects on these cells.

How does vitamin D influence the risk of breast cancer?

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Multiple epidemiological studies have shown that lower circulating levels of vitamin D are associated with an increased risk of breast cancer. The major plasma form of vitamin D is 25(OH)D3 (25D), the pro-hormone, which can be activated by hydroxylation to the active hormone 1,25(OH)2D3 (1,25D). To provide a molecular explanation for the epidemiological observations we have utilised a short-term ex vivo incubation system of human normal and cancerous breast tissues. This approach preserves the tissues 3D structure and allows short-term treatment with 25D or 1,25D. In common with many tissues, breast epithelial cells can metabolise 25D to the active hormone as they can express the hydroxylase CYP27B1. In such tissues there is also rapid induction of CYP24A1 which converts 1,25D to an inactive form.

Using such ex vivo normal and breast cancer tissues treated with 1,25D we isolated RNA and used mRNA-Seq to define vitamin D regulated genes. The success of vitamin D treatment was shown by the induction of highly up-regulated CYP24A1. Only 14 genes were differentially expressed in common between normal and cancer tissue. On considering expression levels and known/possible functions we selected the over-expressed genes KLK6, CLMN, SERPINB1, and EFTUD1 for further analysis. Analysis of expression by qRT-PCR in additional human breast samples treated with 1,25D further confirmed their expression was up-regulated following 1,25D treatment.

Induction of these four target genes by 1,25D are VDR dependent as VDR knockdown in non-malignant breast cell lines resulted in loss of 1,25D up-regulation. From epidemiological studies high vitamin D levels are associated with a lower risk of breast cancer, and therefore target genes induced by 1,25D would be predicted to have tumour suppressor-like characteristics. The known functions of these genes are consistent with this proposed role. In addition, analysis of publically available expression data in breast cancer shows high expression of KLK6, CLMN and SERPINA1 is significantly associated with a more favourable prognosis, particularly in the poorer prognostic triple negative subgroup. These data suggests high levels of vitamin D in breast tissues maintain the expression of specific cancer tumour suppressor genes.


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Remarkable developments in understanding the physiology and pathophysiology of bone have led to new treatments. RANKL was identified as a key mediator of osteoclast development and function in vitro and in vivo. RANKL expression was observed in a species of benign bone tumour, giant cell tumour of bone (GCTB). The use of denosumab, a fully monoclonal antibody to human RANKL, to treat unresectable, locally advanced or metastatic GCTB was first reported in 2010. Since then, confirmatory studies have indicated that denosumab provides long-term disease control for a subset of patients with few other options.
There has been interest in the use of denosumab as an adjuvant to curative surgery, with unclear results to date. More recently another benign connective tissue tumor, tenosynovial giant cell tumor (TGCT), has been targeted with agents blocking CSF-1 signalling, with promising results. TGCT is characterised by a translocation involving CSF-1 and the COIL6A3 gene, resulting in dependence on CSF-1 signalling. These studies point to an important emerging theme in oncology—the use of targeted therapies to treat benign neoplasms. Here I will discuss important implications for clinical trials design, not previously encountered in oncology. These include defining meaningful outcomes, whether there is a role for adjuvant therapy, and the long-term complications of blocking important signalling pathways.

### Low endogenous testosterone levels increase the risk of type 2 diabetes in men, independent of established risk factors

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Background: Limited evidence suggests that low testosterone level may be associated with the development of type 2 diabetes in men.

Aim: To determine the additive predictive value of endogenous testosterone level for the development of type 2 diabetes in men, independent of known diabetes risk factors.

Methods: Data was retrieved from The Men Androgen Inflammation Lifestyle Environment and Stress (MAILES) Study which comprises of two representative longitudinal cohort studies of community-dwelling men aged 35 to 80 years from Adelaide, South Australia. Out of 2563 men, 2101 had a second assessment. 431 cases were excluded for having type 1 or 2 diabetes at baseline and 1670 men were selected. Primary outcome was incident type 2 diabetes. Secondary outcomes include risk stratification by baseline testosterone levels, in relation to waist circumference and age.

Results: 148 men (8.9%) developed type 2 diabetes. Low levels of total testosterone (<18 nmol/L) were associated with significantly increased risk of incident diabetes in an exponential relationship: [Total testosterone: 15-17.9 nmol/L (OR 1.7, 95% CI 1.0-2.8), 8-14.9 nmol/L (OR 2.5, 95% CI 1.7-3.9), 0.1-7.9 nmol/L (OR 7.2, 95% CI 3.3–15.7)]. After adjustment for traditional risk factors and baseline SHBG levels, mild testosterone deficiency (15 -17.9 nmol/L) was no longer associated with higher risk but men with moderate to severely low testosterone levels remained at significant risk: [Total testosterone: 8-14.9 nmol/L (OR 1.9, 95% CI 1.1-3.3), 0.1-7.9 nmol/L (OR 5.8, 95% CI 2.4-14.0)]. Both higher waist circumference (>98 cm) and younger age (<50 years) were highly predictive of incident diabetes in men across the range of low testosterone levels, particularly in those with severe deficiency.

Conclusion: Low testosterone level is an independent predictor of incident type 2 diabetes in men. Younger men with very low testosterone levels and high waist circumference are at greatest risk.

### Lower circulating testosterone (T) is a consequence rather than a cause of poor health in older men: the Concord Health and Ageing in Men Project (CHAMP)

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Aims: Low circulating T in older men is associated with many health problems. We compared cross-sectional and longitudinal analyses of hormones and morbidity in the CHAMP cohort to deduce the direction of causality.

Methods: The population-based CHAMP cohort of men aged 70 years and older were assessed at baseline (n=1705), 2-year (n=1367) and 5-year (n=958) follow-up. At each visit, serum T, dihydrotestosterone (DHT), estradiol (E2) and estrone (E1) were measured by liquid chromatography-tandem mass spectrometry and related cross-sectionally or longitudinally using general estimating equations to self-rated health, quality of life, functional disability, muscle mass and strength, metabolic syndrome, sexual function, bone mineral density, falls and fractures, and cognitive function.

Results: Cross-sectionally, low serum T, DHT, E2 and E1 were associated with most outcomes. Longitudinally, low baseline serum T and E2 predict increased functional disability but no other studied health outcomes whereas low baseline serum E1 predicted deterioration in self-rated health, functional abilities and bone loss. However, a decline in serum T (<10%) or E1 was significantly associated with declines in sexual and cognitive functions over time. As placebo-controlled trials show that (a) the decrease in serum T is too small to explain the decrease in sexual function and (b) testosterone treatment does not improve cognitive function. The decrease in circulating T is more likely to result from, rather than cause, reduced sexual function or cognition.

Conclusions: These findings from a large representative group of older Australian men suggest that declines in serum T levels may be a consequence, rather than a cause, of poor health in older men. Further studies are warranted to investigate serum E1 in men as an important novel health biomarker.
Sex hormone binding globulin and free testosterone as predictors of mortality in men with type 2 diabetes

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5. Objective: To investigate whether the prognostic role of testosterone in men with type 2 diabetes is influenced by its carrier, sex hormone binding globulin (SHBG).

Research Design and Methods: 531 men with type 2 diabetes presenting to a diabetes clinic in 2004–2005 were followed prospectively until death, or July 31, 2014, and a survival analysis was performed.

Results: Over a median follow up mean of 8.8 years (interquartile range 7.3–9.1) 175 men (33%) died. In Cox proportional hazard models both higher SHBG (Hazard Ratio (HR) 1.012 [95% Confidence Interval (CI) 1.002–1.022], p=0.02) and lower calculated free testosterone (cFT) (HR 0.995 [95% CI 0.993–0.998], p=0.001) predicted all cause mortality independently of age, body mass index, presence of macro- and microvascular disease, hemoglobin, renal function, insulin use, and HOMA-IR. By contrast, the inverse association of total testosterone (TT) with mortality weakened after adjustments (p=0.11). SHBG remained predictive (P<0.001) both if substituted for or added to TT in the multivariable model. In the fully adjusted model, an increase of SHBG of 10 nmol/L increased mortality by 12% and a decrease in cFT by 10 pmol/L increased mortality by 5%.

Conclusions: In men with type 2 diabetes, high SHBG and low free testosterone levels proved strong predictors of death, independent of competing mortality factors and of patient characteristics influencing the circulating levels of these hormones. Whether SHBG acts via regulation of testosterone, has intrinsic biological roles, or is a marker of poor health requires further study.

Proportion of undercarboxylated osteocalcin and serum P1NP predict incidence of myocardial infarction in older men.

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Introduction and aims
Undercarboxylated osteocalcin (ucOC) modulates insulin secretion and sensitivity in mice, and higher ucOC is associated with lower diabetes risk in men (1). Diabetes is associated with cardiovascular risk, but the influence of ucOC on incidence of cardiovascular events is unclear. We examined associations of ucOC and other bone turnover markers with incidence of myocardial infarction (MI) and stroke in older men.

Participants and methods
This was a longitudinal study of community-dwelling men aged 70–89 years resident in Perth, Western Australia. Serum total osteocalcin (TOC), N-terminal propeptide of type I collagen (P1NP) and collagen type I C-terminal cross-linked telopeptide (CTX) were measured by immunoassay, and ucOC by hydroxyapatite binding. The ratio ucOC/TOC was calculated. Hospital admissions and deaths from myocardial infarction (MI) and stroke were ascertained.

Results
There were 3,384 men followed for 7.0 years during which 293 experienced an MI, 251 stroke and 2,840 neither. In multivariate analyses, higher ratio of ucOC/TOC (expressed as %) was associated with lower incidence of MI (quartiles Q2-4, ≥49% vs Q1, <49%, hazard ratio [HR]=0.70, 95% confidence interval [CI]=0.54-0.91, but not of stroke (0.99, 0.73-1.34). Higher P1NP was associated with higher incidence of MI (Q2-4, ≥28.2 µg/L vs Q1, <28.2 µg/L, HR=1.45, 95% CI=1.06-1.97), but not of stroke (0.94, 0.70-1.26). CTX was not associated with incident MI or stroke. These results were unchanged excluding enrolment of men experiencing MI within the first year of follow-up.

Conclusions
A reduced proportion of ucOC relative to TOC, or higher P1NP, is associated with increased incidence of MI. UcOC/TOC ratio and P1NP predict risk of MI but not stroke, in a manner distinct from CTX. Further studies are needed to investigate potential mechanisms by which bone turnover markers related to metabolic risk and to collagen formation could modulate cardiovascular risk.


Treating Type 1 Diabetes with Glucocorticosteroids: A case report of PD-1 Receptor inhibition induced Type 1 Diabetes

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The development of immunotherapy has provided patients with metastatic melanoma with improved tumour response and overall survival rates. However, these medications are associated with unique adverse event profiles, unlike other chemotherapeutic agents.

A 61year male presented in diabetic ketoacidosis (DKA) following sequential therapy with ipilimumab and pembrolizumab for the treatment of metastatic melanoma. He had completed four cycles of ipilimumab and had commenced pembrolizumab following disease progression six weeks prior to his presentation. There was no past history or family history of Type 1 diabetes or other autoimmune conditions. He was managed according to a standard DKA protocol and subsequently changed to a basal bolus insulin regimen. Antibodies to GAD or IA-2 were negative and C-peptide was 57pmol/L (300-2350pmmol/L). There was biochemical evidence of mild hyperthyroidism (TSH 0.01, T4 26.4) with negative thyroid antibodies. He had normal pituitary function. He was 2 µg/L, HR=1.45, 95% CI=1.06-1.97.

The IRAE’s are typically managed with high doses of GCS and are mostly reversible. There are only a handful of case reports of pembrolizumab induced T1DM. Beta cell recovery following administration of GCS has not previously been described.

Here we describe a case of DKA in a patient receiving novel immunotherapy for metastatic melanoma. Glucocorticosteroids were used in an attempt to reverse the islet cell IRAE.

Like mother like son? Variable expression and phenotype of an inactivating dominant ATP-binding cassette sub-family C member 8 (ABCC8) gene mutation within a single family.

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Background
Congenital hyperinsulinism (CHI) comprises a heterogenous group of rare disorders characterised by inappropriate insulin secretion secondary to mutations in several genes. Inactivating mutations in the ABCC8 and KCNJ11 genes (encoding for sulphonylurea receptor 1 (SUR1) and K+ inward-rectifying (Kir6.2) subunits respectively of the ATP-sensitive potassium channel on the pancreatic beta cell), account for 40-45% of cases. Disease-causing ABCC8 mutations may be inherited in an autosomal recessive or dominant fashion. Biallelic recessively-inherited disease is more common and causes severe, medically unresponsive disease. Monoallelic (dominant) disease is variable in presentation depending on the specific mutation; most cases are mild although rarer cases of medically-unresponsive disease have been reported. Even amongst patients with identical mutations, expression can be variable. We present a case of an identical dominant ABCC8 mutation harboured by mother and son, with different phenotypic expression.

Case
A 26 year old Caucasian woman (G1P0) was referred for assessment of possible CHI. She’d given birth to a large-for-gestational age (2630g) male infant at 34 weeks gestation, who had required dextrose infusions for severe neonatal hypoglycaemia. Molecular genetic testing detected a dominant missense ABCC8 mutation (p.Arg521Gln) and he was diagnosed with diazoxide-responsive CHI. Parental testing confirmed maternal inheritance, our patient found to be heterozygous for the same mutation. Her history was significant for temporal lobe epilepsy; we wondered whether these episodes were in fact previously unrecognised hypoglycaemia. Multiple attempts to document fasting hypoglycaemia (including continuous glucose monitoring and a supervised fast) revealed no evidence of endogenous hyperinsulinism. Her fast showed appropriate ketogenesis with suppression of insulin and C-peptide – in stark contrast to her son. We find no objective evidence for hypoglycaemia in this patient, who appears to exhibit normal regulation of insulin secretion despite an identical ABCC8 mutation to a proband with severe hypoglycaemia and clearly disordered beta cell regulation.


Empowerment of the next generation of clinicians and researchers in Endocrinology: What are the challenges? The inaugural joint US Endocrine and ESA symposium.
Helena Teede

Young clinicians and clinical and research scientists in endocrinology will be the future lifeblood of our society and field over the next several decades. We have seen major changes in the key focus areas in clinical endocrinology with an aging population, challenges obtaining public hospital positions, a shift in the gender of the workforce, increased engagement in research training (~7000 PhD’s in Australia annually) and falling research funding. In this context the role of recruitment, retention, career opportunity and development and empowerment of young clinicians and researchers in endocrinology are important. In this session past and current US Endocrine Society and ESA President’s will explore these themes in the US and Australian context. We will also discuss career challenges for young women and men trying to balance career and family. The session will be interactive and include a panel discussion with younger ESA members and will assist the society in strategic planning, lobbying and progressing the future of this field.

Title: Empowering the next generation of endocrinologists: United States perspective
Richard Santen

Basic and clinical research is the lifeblood of endocrinology and necessary to enable the field to innovate. Research requires funding; the monies to support young investigators are diminishing and being shifted to those more senior. The average age of an investigator to compete successfully for his/her first independent grant has increased from 36 in 1980 to 45 today. The percent of investigators over age 66 has increased from 0.2% to 7%. In 1962 57.9% of submitted grants were funded and the percent now is approximately 14%. At the same time the total dollar support from the NIH has decreased by 28% from 2003 to 2013 when adjusted for inflation. These statistics emphasize how challenged a young investigator can be in competing successfully for funding of his/her work. Other challenges are the competing responsibilities incurred when working in an academic institution. Basic scientists spend 17.7% of time on administration, 7.6% on clinical practice, and only 59.1% on research. Clinician scientists spend 12.4% of time on administration, 27.9 % on clinical practice and 43.5% on research. Many projects require nearly full time in the laboratory and this is not possible unless the work week is extended to 80-100 hours. For clinicians, the challenges in the United States are to meet the increasingly stringent requirements for documentation and the very short time allowed to see new and follow-up patients (generally 40 and 20 minutes). All of these challenges have discouraged young professionals from going into endocrinology as a field. It is estimated that 70% of endocrinologists in training programs in the USA currently are foreign medical school graduates, a major change from 20 years ago. The method in which research is conducted is changing. With the depth and breadth of results needed to be competitive, multidisciplinary groups are required in which investigators are inter-dependent, not independent.

What can be done to empower the next-generation of endocrinologists? The Endocrine Society recently empaneled a task-force to address this issue. Key recommendations included: (1) work with training program directors and mentors to educate about professional development possibilities (2) highlight the satisfaction of endocrinologists in their work through testimonials and outcome data (3) incorporate Nex-Gen members into society taskforces, committees and governance structure (4) develop mock study sessions and grant review processes targeted at young endocrinologists (5) create online educational materials targeted at Nex-Gen members (6) initiate a comprehensive career
development program (7) Enhance Nex-Gen visibility by instituting a series of regional meetings with platform presentations and by appointing Nex-Gen members as annual meeting session chairs paired with more senior investigators (8) create informational tools to summarize all funding opportunities (9) establish career development sessions analogous to meet the professor sessions. In summary, only by a comprehensive approach which addresses all of the needs of Nex-Gen members can we overcome a potential crisis in the future where our societies become predominantly composed of older members.

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Understanding how maternal obesity and fetal neuro-immune interactions change the development of the hypothalamic arcuate nucleus in the mouse

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A mother’s obesity during pregnancy is well-recognised for its ability to elevate her offspring’s risk of obesity and the metabolic syndrome. Because the physiology of the mother and her offspring interact most intimately during gestation, we have been characterising the changes that take place to the fetal hypothalamus during its development in an obese mother.

Female C57B/6J mice were fed a high-fat diet (45% kcal from fat) for 6 weeks, which results in >30% increase in body weight compared to control-fed age-matched females; and offspring show elevated body weight. At birth, a significant reduction was observed in the neuronal connectivity between the arcuate nucleus (ARC) and the paraventricular nucleus of the hypothalamus (PVN), suggesting a developmental anomaly in axon growth from ARC to PVN. Transcriptome analysis was performed on fetal ARC to determine whether anatomical changes could be accounted for by altered developmental gene expression. Significant changes were observed in the expression of two genes – DCC and Unc5d – encoding receptors for the axon growth and guidance ligand, Netrin-1. Both DCC and Unc5d were expressed by body weight regulating neurons in the ARC during gestation; and exposure of ARC neurons to Netrin-1 in vitro stimulated growth cone expansion and branching.

To identify candidate factors linking maternal obesity to altered developmental gene expression, a multiplex cytokine assay was used. This revealed significantly elevated interleukin-6 (IL6), IL17A, and interferon gamma in the fetal circulation at gestational day 17.5. Exposure of ARC neurons to IL6 in vitro abolished their ability to respond to Netrin-1.

Together these data suggest that disruption of normal Netrin-1 signaling consequent to aberrant cytokine exposure may account for altered neuronal connectivity of ARC neurons in fetuses undergoing gestation in obese dams.

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New technologies to resolve old questions : Using optogenetics to elucidate GnRH/LH pulses

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The reproductive system is critically dependent upon pulsatile hormone release, which is driven by a small population of gonadotropin-releasing hormone (GnRH) neurons located in the hypothalamus. In particular, the secretion of luteinizing hormone from the anterior pituitary, is essential for reproduction and tightly patterned and controlled by the secretion of GnRH.

Until recently, the scattered distribution of the GnRH cell bodies limited the investigation of the cellular events that lead to pulsatile secretion of LH. Therefore, the numbers of GnRH neurons involved in a pulse, their location, and patterns of electrical firing had never been determined. Using cutting edge optogenetic technology, we generated a mouse model in which the GnRH neurons that control gonadotropin secretion could selectively be activated in living animals using blue light delivered to the hypothalamus using optical fibres. Previously we have characterized the profile of pulsatile secretion of LH in ovariectomized conscious mice using a fast blood sampling collection and here aimed to replicate these LH pulses in vivo. Using a range of different frequencies and durations of optogenetic stimulation, we have been able to define that 10 Hz stimulation for 2 min was sufficient to generate a pulse-like increment of LH release. The same result was found for optical activation of GnRH projections in the median eminence. Under these conditions, the dynamics of optogenetically-evoked LH pulses paralleled to that of endogenous LH pulses suggesting that the minimal parameters of GnRH neuron activation we found were likely to closely resemble the dynamics occurring in vivo. This first insight into how GnRH neurons generate a pulse of LH in vivo provides critical information for understanding and manipulating the genesis of gonadotropin pulsatility in reproductive biology.

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Neurodegeneration: an endocrine and metabolic perspective

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Motor Neuron Disease (MND) is a fatal neurodegenerative disease for which there is no cure. For most patients, death occurs within 2–5 years of diagnosis. While the primary pathology in MND is the death of motor neurons, there is evidence that defective energy metabolism affects disease pathogenesis. Moreover, increased body mass index and high calorie supplementation is associated with improved prognosis in MND patients. Thus, we hypothesise that in MND, the state of metabolic flux modifies disease course, and modulation of metabolic homeostasis will aid in sustaining survival. We have studied mouse models and human subjects to investigate how altered metabolic homeostasis impacts disease outcome.

During this talk, I will present data that demonstrates key endocrine and physiological adaptations that facilitate the mobilisation and uptake of energy substrates to meet increased metabolic demand. Maintenance of optimal energy supply plays an integral role in slowing disease progression and sustaining survival in MND. Our studies not only establish the disease-promoting role of altered energy metabolism in MND, but also pave the way for future clinical testing of novel therapeutics aimed at improving metabolic capacity to prevent the death of neurons and muscle in neurodegenerative diseases.

N/A

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Content not available

Curing preeclampsia: beyond blood pressure control

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For more than half a century “treating” hypertension to prolong pregnancy for better fetal maturity has been the core of managing preeclampsia. The recognition, most effectively forwarded by Jim Roberts and Chris Redman more than 20 years ago, that maternal endothelial dysfunction secondary to placental “factors” fundamentally underlies the maternal features of preeclampsia heralded opportunities for new approaches to management. Since that time our understanding of the vasoactive “factors” released by the placenta and the endothelial cell pathways disturbed by those factors have advanced significantly. Indeed, the field is now poised for significant new treatment strategies, for both placental and endothelial cell targets, such that a secondary “cure” for preeclampsia is, at last, a genuine prospect.

We have identified pathways within the endothelial cell (EC) that offer promise of targeted therapies at different points. Using our example of activin-NADP(H) oxidase-EC dysfunction, examples of novel therapeutic targets will be discussed and explored, including optimising cellular anti-oxidant therapy and stabilising EC function. The importance of animal models of preeclampsia in informing best design for clinical interventions will also be explored, emphasising the critical role of experimental research in advancing what is a uniquely a clinical human disease.

A systematic screening approach to identify therapeutics for preeclampsia

Stephen Tong

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Preeclampsia is a major complication of pregnancy. Responsible for thousands of maternal and fetal deaths, there is no treatment to arrest disease progression, except delivery. Therefore, a therapeutic could substantially improve fetal and maternal outcomes.

The preeclamptic placenta releases the anti-angiogenic factors soluble fms-like tyrosine kinase 1 (sFlt-1) and soluble endoglin (sEng) into the maternal circulation, causing endothelial dysfunction and organ injury. Also, preeclampsia is strongly associated with oxidative stress. Therefore, an ideal candidate therapeutic would be a drug that is safe in pregnancy and can do the following: 1) decrease sFlt-1 and sEng secretion 2) block endothelial dysfunction 3) up-regulate endogenous anti-oxidant defences and 4) is effective in an animal model of preeclampsia.

Over two years, our team has developed a preclinical screening pipeline to identify drugs that are safe in pregnancy and have these important biological actions. Importantly, we only use primary human tissues.

For example, we have found proton pump inhibitors (e.g. esomeprazole, they are commonly used to treat gastric reflux that occurs during pregnancy) are a candidate treatment. They have potent effects in 1) decreasing sFlt/sEng expression and secretion from 4 different primary tissues (primary trophoblast, preeclamptic placental explants, two endothelial cell types), 2) blocking endothelial dysfunction (five different assays, including whole vessel pressure myography) and 3) up-regulating anti-oxidant defences. Furthermore, proton pump inhibitors 4) ameliorate the preeclamptic phenotype in an animal model where
sFlt-1 is over-expressed in the placenta. Consequently, we have moved this concept into a phase II randomised clinical trial, about to commence in South Africa.

Using this approach to screen for candidate therapeutics for preeclampsia, we have validated the premise that pravastatin may be a treatment. In addition, we identified other exciting candidate treatments, including metformin, solfacone, YC-1, sulfasalazine, and even epidermal growth factor.

Clinical Management of Pre-eclampsia
Mark Brown

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The clinical management, and therefore pregnancy outcome for women with pre-eclampsia begins with clear recognition that the disorder is arising. Living in Australia we are fortunate that antenatal care affords this opportunity in most cases and management can be effective. World-wide pre-eclampsia is responsible for about 75,000 maternal and half a million perinatal deaths per year, mortality about 40% as great as that of HIV.

Initial recognition still relies upon detection of hypertension or proteinuria, though at least 25% of cases are non-proteinuric, now recognised by most societies world-wide. Blood pressure is best measured in pre-eclamptic women by the auscultatory method using a liquid crystal sphygmomanometer rather than automated devices. Proteinuria can be immediately assessed by a spot protein/creatinine ratio and 24hr urine tests are not required. Future diagnoses may include measures of angiogenic or other factors but these should not be employed in clinical practice yet.

There is increasing support for a conservative approach in women presenting pre-term, waiting for a clear indication for delivery in these cases. RCT evidence confirms that controlling maternal BP to a target diastolic BP of 85mmHg is associated with fewer episodes of severe maternal hypertension.

IPD analysis of almost 100,000 women has confirmed a recurrence risk of 16% in future pregnancies, more likely if delivery was early in the index pregnancy. Recent surveys have found that physicians are less likely than obstetricians to appreciate the long-term cardiovascular risks of pre-eclampsia. These women have post-partum subtle features of metabolic syndrome and elevated BP but often remain untreated due to comparison of their results against usually older cohorts. Our current studies aim to determine normal physiological and BP limits for young parous women in order to help detect more subtle abnormalities in women who have had pre-eclampsia.

Understanding of early-onset preeclampsia through a placenta-specific protease
Guiying Nie

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Preeclampsia (PE) is a life-threatening complication of pregnancy; it can affect multiple organs in the mother leading to renal failure, abnormal liver function, haemolysis, seizures, severe oedema and stroke. Despite worldwide research for many years, there is no effective “cure” for PE. The current treatment is to deliver the baby, often prematurely, to save the mother’s life. Although wide-spread and multi-organ endothelial dysfunction seems to unify PE symptoms, it is increasingly recognized that PE is not a single disease but a complex syndrome. To find treatment for PE, we need to study PE subtypes and search for subtype-specific treatments.

The field is still defining how to best subtype PE. One classification is based on the timing of disease presentation, early (<34 gestation weeks) and late (>34 weeks) onset. Early-onset PE is often associated with severe disease and intrauterine growth restriction (IUGR), and requires premature delivery. Late-onset PE is rarely linked to IUGR but can also be severe. It is also suggested that early-onset PE is primarily of placental origin, whereas late-onset PE is more related to maternal failure of adaptation to pregnancy.

This talk will focus on our recent research on a serine protease that is expressed only by the placenta and significantly up-regulated in early-onset PE. Our data strongly suggests that this protease, which is of placenta-origin but circulating in the maternal blood, disturbs maternal vascular homeostasis and contributes to the development of early-onset PE. There also appears a direct link between this protease and IUGR. Our studies indicate that this protease has potential for the development of treatment for early-onset PE.

Metabolism strikes back: metabolic flux regulates cell signalling and proliferation
Evan Simpson

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Over 80 years ago, Otto Warburg showed that many tumour cells displayed a high rate of aerobic glycolysis, and he suggested that ‘this was the cause of cancer’. This concept lay fallow for decades, as interest in metabolism faded with the rise of recombinant DNA technology. Certainly this Warburg Effect correlates with proliferative capacity and is the basis of PET scanning, but it is only in the last decade or so that interest in metabolism has revived with the development of techniques to...
study its regulation. But it has been generally assumed that these changes in metabolism follow the changes in gene expression. However, there is increasing evidence that the reverse is true, namely that metabolic flux is a driver of gene expression, leading Hanahan and Weinberg to state that metabolism should be considered as one of the Hallmarks of Cancer (Cell, 2011).

SERPINA1 is a direct Estrogen Receptor target gene and a predictor of survival in breast cancer patients

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Publish consent withheld

Genetically Modified Mouse Models to Dissect the Physiological Roles of ERα's Functional Domains

Kenneth Korach1, Y. Arao1, K.J. Hamilton1, L. Coons1, S.C. Hewitt1

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Estrogenic biological effects are primarily mediated through the two estrogen receptor isoforms (ERα and ERβ). Three main mechanisms of action for ERα include the nuclear genomic actions, including the classical and "tethered"- mediated responses, and the non-nuclear, non-genomic, rapid action responses. Liganded ERα binds to estrogen responsive elements of target genes directly (classical) or "tethered" with Sp1 or AP1 family members to activate gene expression. The non-nuclear ERα rapid response signaling activity includes different actions as exemplified by cellular calcium mobilization, nitric oxide synthesis, and activation of signaling cascades. In order to mediate these varied activities, ERα, like all nuclear receptors, maintains the classical domain demarcations that contain different functionality, such as Activation Function -1 (AF-1), AF-2, DNA binding domain (DBD), ligand binding domain (LBD) and nuclear localization signal (NLS). Understanding the in vivo actions of these functional domains has been delineated primarily through in vitro cell culture systems. To dissect the physiological functionality we have generated different ERα mutant mouse models. Classical ERα mediated DNA responses can be abrogated by a double point mutation in the DBD (K/KO mice) and in this mouse model, only "tethered" and rapid action responses are present, the K/KO action corrects some effects, but does not rescue αERKO phenotypes of the uterus and mammary gland. AF-2 is the ligand activated transcription function assigned to helix 12 of the LBD. Point mutations in this region no longer allows for estradiol dependent ERα activation, but responds agonistically through AF-1 to the ERα antagonist, ICI182780 and to some SERM's, but others remain antagonists (Raloxifene, basodioxifene). We have generated an AF-2 mutant mouse (AF2ER mouse) to dissect the in vivo physiological functions of AF-1 and AF-2. The AF2ER mouse shows phenotypes similar to the αERKO with male and female infertility and lack of hormonal responses consistent with the estradiol inactivity and ligand independent activation. Treatment in vivo with ICI or TAM results in differential tissue activities of the AF2ER mutant receptor suggesting that ERα using AF-1 and AF-2 in different tissues to mediate its actions. Understanding the tissue selective AF-1/AF-2 utilization should provide further insight for the potential to develop tissue selective hormonal therapeutics.

Dysregulated metabolism in obesity and breast cancer: Clues to novel therapeutics strategies?

Kristy Brown

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Obesity is a known risk factor for estrogen-dependent postmenopausal breast cancer. Factors, including adipokines and inflammatory mediators (e.g. prostaglandin E2; PGE2), converge to increase aromatase expression, and hence estrogen production, within the adipose tissue of the breast, thereby increasing the risk of estrogen-dependent breast cancers. We have previously shown that the metabolic pathway involving LKB1/AMPK, as well as the AMPK-regulated tumour suppressor p53, are inhibitory of aromatase expression, and that factors produced in obesity inhibit these pathways. We have recently demonstrated that p53 is a negative regulator of aromatase, that p53 expression and phosphorylation are inhibited by PGE2, and that there is a positive correlation between inactive p53 and aromatase in clinical samples of breast cancer. Conversely, we have showed that HIF1α is a potent stimulator of aromatase PI1, and that it is stimulated by PGE2 and positively associated with aromatase expression in breast tumors. HIF1α is best characterized for its role in mediating responses to hypoxia and is negatively regulated by AMPK. These studies have also led us to explore whether other factors regulated in obesity may be involved in regulating aromatase in the breast. We have recently demonstrated that the gut hormone ghrelin, known to be inversely associated with obesity and a strong stimulator of AMPK, and its unacylated form, des-acyl ghrelin, are potent inhibitors of aromatase at picomolar concentrations. We also find that des-acyl ghrelin inhibits the estrogen-dependent growth of breast cancer cells in vitro and in vivo.
These findings therefore support a role for dysregulated metabolism as a driver of aromatase expression, and provide an additional molecular link between obesity and breast cancer in older women. The identification of these new molecular links has led to new therapeutic strategies, which we are currently exploring in preclinical and clinical studies.

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**New windows into the body**

**Mark Hutchinson**

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The Australia Research Council Centre of Excellence for Nanoscale BioPhotonics is a $40M investment by the ARC, state governments, academic institutions and industry to explore novel light based sensing technologies that are to create "windows into the body" to sense the previously unsensable. How many new terms can we use in the first sentence of a conference abstract? Apparently lots! But if you are interested, then you will love this session, because the basic understanding of the principals of these novel sensing modalities will be introduced and explained. Importantly, the relevance of these technologies and experimental approaches have for biology will be explored.

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**Through the looking glass: what can we see in the early embryo when we look carefully enough**

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I will explain how to use subtle properties of cell colour to provide information about cell biochemistry. Crucially, the method is label free, avoiding biochemical interference with cells. Our methodology is able to detect endogenous metabolites in biological specimens in a variety cells and tissue with highly discriminative results. The autofluorescent emission from these samples represent a mixture of spectral signatures from these compounds, measured under a range of specifically chosen excitation wavelengths. These are employed to generate a useful and biologically significant feature sets from which we can extract two or three highly informative feature variables highlighting the most interesting aspects of the data. Further we identify the abundance of these compounds at each pixel. We analyse all acquired cell images in a fully automated way without subjective choices. The process aims to derive maximum quantitative information from cell images, and statistics is used whenever required to assess population properties and differences.

I will present selected examples of applicability of this quantitative colour imaging method (1) for differentiation of cells and for biochemically mapping key fluorophores in cell populations, including statistics of fluorophore content; (2) for the detection of cell subpopulations answering the question of whether cells form distinguishable clusters; (4) for finding label-free signatures of cell subpopulations, which demonstrate that, in some cases, antibody labeling may in some cases be replaced by measurements and analysis of autofluorescent characteristics. These methods have been applied to several cell types including olfactory neuronal cells, adipose-derived stem cells before and after osteogenic differentiation, induced pluripotent stem cells (ips) cells, motor neurone disease cells, various cancer cells (MCF10A and MCF7) and diabetic tissue. An example, in cells with a mitochondrial mutation, cellular maps of native fluorophores, flavins, bound and free NADH and retinoids unveiled subtle metabolic signatures and helped uncover medically significant cell subpopulations, in particular, a subpopulation with simultaneous low bound NADH, high free NADH and high lipofuscin content.

I will also show the application of this new type of label-free imaging and image analysis to the early embryos.

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**Nanodiamond for BioPhotonic and Hybrid-Photonic applications**

**Brant Gibson**

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Fluorescent nanodiamonds (NDs) have a range of unique properties which make them highly desirable for bioimaging and biosensing applications. Their fluorescence is produced via optical excitation of atomic defects, such as the negatively charged nitrogen vacancy centre, within the diamond crystal lattice. Possessing long-wavelength emission, high brightness, no photobleaching, no photoblinking, single photon emission at room temperature, nanometer size, biocompatibility, and an exceptional resistance to chemical degradation make NDs almost the ideal fluorescent bioimaging nanoprobe. I will discuss these exciting properties in detail and also give some examples of their integration with photonic materials for future hybrid ND-biophotonic applications. In addition, I will discuss details of our custom confocal fluorescence microscopes and also show some examples of bio-images which have been captured on the systems.
Microstructured Optical Fibers and Photoswitches: Light-Driven Sensors for Metal Ions in Biology.

Sabrina Heng¹

¹1. Adelaide University, Adelaide, SA, Australia

Given the intricate relationship between metal ions and reproductive biology, there is need to develop new metal ion sensors that can provide rapid/real-time information, are reusable and capable of continuous or repeated measurements. In this context, sensors with 'photoswitchable' properties where ion sensing can be turned 'On' and 'Off' with different wavelengths of light, would allow for multiple measurements to be made on a single sample without the need to change the probe. This is a highly desirable property in biological experiments, where sample availability and volumes often limit the number of experiments that can be performed. This talk highlights the recent advances made by our group in developing nanoliter-scalerenergicreable ion sensors based on microstructured optical fiber (MOF). Here, the air holes of the MOF are functionalized with a specific photochromic molecule, to yield a switchable sensor that can detect metal ions such as zinc and calcium, down to nanoliter-scale volumes, where ion binding is turned on and off upon irradiation with light. Unbound ions are readily flushed from the fiber in the 'off' state to allow the sensor to be reused. The integration of an ionophore into the sensor paves the way for the development of highly specific light-based sensing platforms that are readily adaptable to sense a particular ion. This work represents advances in both fiber sensing technology and indeveloping new tools for answering biologically related questions, particularly in the areas of reproductive health.

Development of optical fibre probes for biosensing applications

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Over the preceding decades optical fibres have expanded beyond their typical use in communications, to alternate applications in active devices and sensors. While the majority of the work in this field has focused on the development of structural health monitoring tools for civil engineering and aviation, an ever increasing interest has developed in the use of optical fibres for biosensing applications.

The unique guiding properties of optical fibres, combined with the ability to place the sensing element has allowed for the use of these fibres in widely varied applications. By adding a functional element to the fibre, typically through the use of a fluorophore layer or resonant feature, the fibre can be sensitized to a particular parameter such as temperature, pH or chemical concentrations.

In this work we discuss the use of both microstructured fibres, where small holes run along the length of the fibre to facilitate interaction of the light with the analyte to be measured, as well as the development of tip-based sensors using conventional large mode area optical fibres.

By functionalizing these fibres sensors capable of performing temporal and spatial measurements on very small volume samples are developed, which show good potential for performing measurements in the local media surrounding embryos which would typically be difficult to analyse using conventional microscopy based techniques. These sensors are capable of performing measurements of the pH, the temperature experienced at a localised spatial position.

A Non-invasive Sensor for Hydrogen Peroxide and pH

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Hydrogen peroxide (H₂O₂) is a reactive oxygen species (ROS) shown to affect the developmental competence of embryos, as do fluctuations in pH. Methods for detection of H₂O₂ and pH often involve the addition of fluorescent dyes and confocal imaging. This poses significant scientific and ethical problems for monitoring of embryos during human in vitro fertilisation (IVF), as the effect of these compounds is unknown and direct contact is unadvisable. Therefore, the non-invasive sensing of H₂O₂ and pH is an important target both for understanding embryonic development and the clinical monitoring of embryo health. This work reports the use of organic fluorophores for the detection of H₂O₂ and pH while embedded in a polymer coating on an optical fibre surface. CarboxyPeroxyfluor-1 (CPF1) and semi-naphthorhodofluor-2 (SNARF2) were immobilised to the fibre tip by a UV-catalysed polymerisation of acrylamide to the optical fibre surface. It is verified here that both H₂O₂ and pH can be
Is immediate surgery really necessary in every patient with primary or recurrent papillary thyroid cancer?

Michael Tuttle

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The traditional approach to the management of differentiated thyroid cancer placed a premium on early detection and immediate intervention for both the primary disease lesion and for biochemical or structural evidence of persistent/recurrent disease. While this aggressive treatment strategy was viable and effective when our rather crude disease detection tools identified primarily large volume macroscopic disease, it is much less clear whether an aggressive approach to either very low risk thyroid cancer primaries or small volume persistent/recurrent disease that is only detected with highly sensitive imaging tools and blood tests is beneficial to patients. Multiple studies now demonstrate that small volume disease in carefully selected patients (both in the primary setting and in the persistent/recurrent disease setting) progresses very slowly in the vast majority of patients and is often not “cured” with additional surgery or radioactive iodine. Furthermore, some of our well meaning treatments may be associated risks that are greater than the potential harm that could be caused by small volume disease. We have reached a point where our highly sensitive detection tools are identifying small volume disease that is very unlikely to cause any harm.

In this lecture, we will re-evaluate what we think we know about the natural history of small volume primary thyroid cancers (papillary microcarcinomas) and small volume minimal residual disease (biochemical incomplete and/or structural incomplete). We will question the clinical benefit, effectiveness, and toxicity of additional therapies aimed at destroying all residual disease and describe a clinical management paradigm in which clinically important disease is identified and appropriately treated while small volume disease that is unlikely to cause immediate harm is followed with active surveillance utilizing a deferred therapeutic management approach.

Screening and autopsy studies indicate that asymptomatic papillary microcarcinomas are present in at least 5-10% of the United States adult population (representing nearly 16 million people with undiagnosed thyroid cancer). Despite this huge pool of subclinical cases, the prevalence of thyroid cancer in United States is only 0.5 million patients indicating that <3% of this subclinical reservoir has been detected and diagnosed. However, the current management paradigm of aggressive disease detection using US guided fine needle aspiration is resulting in a dramatic increase in the identification and therapy of very low risk thyroid cancers.

Interestingly, data from our Japanese colleagues indicates that the vast majority of these subclinical thyroid cancer foci progress either slowly or not while under observation with serial ultrasound examinations. Importantly, even the small number of patients that demonstrated disease progression while under active surveillance were effectively treated with thyroid surgery indicating that a delayed surgical management approach in properly selected patients had no impact on disease specific survival.

In light of the very low disease specific mortality associated with papillary microcarcinomas, the lack of proven benefit of thyroid surgery, and the potential for side effects and complications from thyroidectomy, we are obligated to carefully consider alternative management strategies. The 2015 American Thyroid Association thyroid cancer management guidelines will state that while surgery is generally recommended for biopsy proven thyroid cancer, an active surveillance management approach “can be considered” as an alternative to immediate surgery in patients with very low risk tumors. Furthermore, the 2015 ATA guidelines strongly discourage FNA of asymptomatic sub-centimeter thyroid nodules, even if ultrasonographically suspicious, endorsing serial ultrasonographic follow-up with cytology evaluation recommended only if there is evidence of disease progression.

Male Infertility: Biomarkers for the diagnosis and future prediction of men's health

Mark Baker

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Male infertility is a very common condition, with reports suggesting that one in 15 men of reproductive age are affected1. The diagnosis of male-factor infertility is difficult and involves discounting female infertility through hormone measurements, pelvic examination and invasive laparoscopy. A semen profile analysis can suggest male infertility, if sperm counts are <15-20 million/ml, or <50% of sperm possess forward progressive motility (and < 25% rapidly progressive sperm) or <4% good morphology sperm. However, for many couples (20-30%), infertility remains largely unexplained.

In addition, infertile men appear to have more than their share of problems. Not only are they dying younger, but on average demonstrate three times the average rates of cancer compared to the general population2. As such, it appears that spermatogenesis may give a “prophetic” insight into the overall health of men.

As such, we have used quantitative proteomics analysis to compare spermatogonia taken from healthy, fertile individuals and compared the proteome to that of an infertile male. Several proteins were found to be altered, including, the sperm specific protein, Outer Dense Fibre 1, which was virtually absent from the gametes of the infertile male. A second cohort of men, were missing the major chromatin compaction protein, MENT, together with Histone H2A Bbd and HSP4AL. Given these proteins are also expressed in somatic cells and regulate chromatin compaction, this represent the first biochemical insight as to how male infertility, may predict the future of a man’s health.
Do intra-uterine androgens play a critical role in preparation for pregnancy in women?

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During the establishment of pregnancy, the endometrium undergoes dynamic remodelling in order to establish a 'receptive' microenvironment. Decidualisation, a key part of this process, is characterised by differentiation of endometrial stromal fibroblasts which secrete growth factors and cytokines that regulate vascular remodelling and immune cell influx, processes that are essential for implantation and placentation development. Recent studies in our laboratory have revealed that decidualisation results in altered expression of enzymes that regulate biosynthesis and metabolism of estrogens. We believe that intra-tissue steroid production may play an important regulatory role in the endometrium during the establishment of pregnancy. In the present study, we tested the hypothesis that changes in the availability of bioactive androgens impact on uterine function during decidualisation. Primary human endometrial stromal cells were isolated from endometrial biopsies collected from women during the proliferative phase of the cycle (n=20). In vitro decidualisation was induced by treatment with progesterone and cAMP. The expression of androgen biosynthetic enzymes was assessed by qPCR, Western Blot and immunocytochemistry. Concentrations of the decidualisation marker IGFBP-1 and testosterone (T) and dihydrotestosterone (DHT) were determined by ELISA. We found that decidualisation was associated with biosynthesis of androgens. Time-dependent changes in the expression of androgen biosynthetic enzymes AKR1C3 and SRD5A1 were detected by qPCR (n=8 patients, p<0.01) and Western blot. Decidualisation was associated with secretion of the androgen receptor agonists T and DHT (n=8, p<0.001). Androgen action was inhibited by co-treatment with the antiandrogen flutamide which significantly reduced secretion of the decidualisation marker IGFBP-1 (n=8, p<0.01) and altered the expression of receptivity genes such as SPP1 (n=8, p<0.001). These data suggest intra-uterine androgens may be critical for decidualisation and endometrial receptivity. We speculate that decidualisation is associated with a unique steroid microenvironment that may play a critical role by 'fine tuning' the endometrium in preparation for pregnancy.

Energy pathway metabolomics in hereditary phaeochromocytomas and paragangliomas

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Phaeochromocytomas and paragangliomas (PPGLs) are highly heterogeneous tumours that based on gene expression profiling fall into two cluster groups: cluster 1 PPGLs are phenotypically immature tumours due to mutations resulting in activation of hypoxia-angiogenic pathways; cluster 2 tumours are more mature adrenaline-producing tumours caused by mutations of genes leading to activation of kinase signalling pathways. Among cluster 1 tumours, those due to mutations in genes encoding succinate dehydrogenase (SDH) subunits and other Krebs cycle energy pathway enzymes are more often malignant than other PPGLs. Tumourigenic mechanisms responsible for SDH-mutated PPGLs involve a block in Krebs cycle energy pathway conversion of succinate to fumarate with resulting highly elevated tumour tissue levels of succinate. The extent of increase in succinate relative to the decrease in fumarate, reflecting the degree of functional impairment of SDH, varies depending on the SDH subunit affected. PPGLs due to SDHB mutations are more prone to malignancy and also exhibit more pronounced impairment of SDH function than PPGLs due to other SDH mutations. Succinate acts as an oncometabolite that inhibits alpha-ketoglutarate-dependent enzymes, including prolyl hydroxylases responsible for proteosomal degradation of hypoxia inducible factors, including the HIF2α expressed in cluster 1 PPGLs. For SDHB-mutated tumours, additional succinate mediated inhibition of DNA methyltransferases also leads to a hypermethylator phenotype and further silencing of genes involved in controlling proliferation. The inverse relationship between differentiation and disease aggressiveness reflects channelling of energy to tumour growth in SDHB-mutated PPGLs. These considerations point to possible therapeutic targets. The block in oxidative phosphorylation at complex II implies a need for alternative energy sources for tumour growth and a possible Achilles' heel for targeted therapies for metastatic PPGLs. Reversing the DNA hypermethylation in SDHB-mutated metastatic PPGLs provides another potential therapeutic strategy. A third strategy involves blockade of HIF2α or its downstream signalling pathways.

References:
2. Eisenberg, M.L., Betts, P et al., 2013. Increased risk of cancer among azoospermic men. Fert. Ster. 100. 681-685
Biomarker discovery and application in Endocrine Cancers

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A good biomarker should assist clinical decision-making – either by stratifying prognosis or identifying therapeutic opportunities. These issues will be highlighted in considering two such immunohistochemistry biomarkers now used routinely in our institution: detection of oncogenic BRAF in thyroid cancer; and detection of SDH deficiency in phaeochromocytomas and paragangliomas.

A majority of thyroid cancers contain somatic gene alterations causing activation of the MAP kinase signaling pathway, of which the commonest is BRAFV600E that occurs in about 60% of papillary thyroid cancer (PTC) cases in Australia. A recent multicenter study found that BRAFV600E was significantly associated with increased cancer-related mortality among patients with PTC. The conundrum in using BRAF as an adverse biomarker is that occurs so commonly in a disease that usually has a good outcome: it has been recently recognized that mortality is better predicted when BRAFV600E is accompanied by mutation in the TERT promoter.

Germline mutations in one of the four genes encoding subunits of succinate dehydrogenase (SDH) are collectively the commonest cause of heritable phaeochromocytoma/paraganglioma syndromes. Loss of SDHB immunostaining has proved to be an important tool for recognising tumours associated with mutations in any of the SDHx genes; loss of SDHA immunostaining is more specific for germline mutation in SDHA. The utility of these biomarkers was emphasized in characterizing SDH-deficient renal cell cancers, gastrointestinal tumours and pituitary adenomas that are also associated with germline SDHx mutations. Moreover, negative SDHB immunohistochemistry provides functional validation of pathogenicity when variants of otherwise uncertain significance are identified in SDHx genes.

Tumour Profiling in Thyroid Cancer

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Publish consent withheld

Endometrial hyperplasia and cancer: Side effects of miscommunication

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The tissue microenvironment, which is mostly comprised of extracellular matrix, adipocytes, blood vessels, immune, and mesenchymal fibroblasts, plays an important role in normal organogenesis, tissue homeostasis, and can lead to carcinogenesis when pathologically disrupted. Tissue recombination studies have shown that mesenchymal signals direct the fate of adjoining epithelial cells. For example, recombination of vaginal epithelium with uterine mesenchyme results in development of uterine epithelium. The importance of the microenvironment in cancer has been reported in human studies showing that paracrine signals from cancer associated fibroblast (CAF) cells play an important role in tumour development, progression and drug resistance. Gene sequencing of breast cancers and endometrial polyps has revealed genetic mutations in the stromal component. However, the contribution of the stromal microenvironment to the progression of endometrial hyperplasia and cancer has not been well explored.

We studied age related changes in mouse and human uteri, and examined their contributions to the development of endometrial hyperplasia and cancer. Our studies revealed that with age, stromal cells of human and mouse uteri progressively express alpha Smooth Muscle Actin, which is not normally expressed in endometrial stromal fibroblasts and suggests that the aged stromal cells have differentiated into the carcinogenic CAF phenotype. Using a co-culture method, we showed that aged fibroblast cells actively promote the growth of human endometrial cancer cells. Proteomic analysis of aged and young uteri revealed higher expression of the growth promoting factors such as VEGF in aged uteri. Collectively, our results have shown the significance of stromal cells in endometrial cancer.

SMS and JG contributed equally to this study
Androgen Action and Ovarian Function in Health and Disease
Kirsty A Walters

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Androgen action mediated via the androgen receptor (AR) is long known as vital for male reproductive development and function but the biological role of androgen action in female reproduction is only now being unravelled. Deciphering the specific mechanisms and precise pathways by which AR-mediated actions impact on ovarian function has been hindered by ambiguity of pharmacological investigations relying on aromatizable androgens that also act via estrogen receptors. Using our novel global and cell-specific female AR knockout mouse models we firmly established a role for AR-mediated androgen actions in ovarian function, maintaining optimal female fertility and are deducing the mechanisms of androgen action in governing follicle health, development and ovulation. Furthermore, observational human studies and animal experiments provide substantial evidence for a role of AR-mediated androgen action in the origins of the most frequent ovarian pathology, polycystic ovarian syndrome (PCOS). We have established an optimal PCOS mouse model and, by combining this model with the female AR knockout mouse models, we have provided strong evidence that AR signaling may be an important initiator of PCOS. In concert, these findings illuminate the key roles of AR-mediated androgen action in the optimizing female fertility and ovarian function, as well as providing novel insights into the mechanisms involved in the androgen-associated reproductive disorder PCOS.

Why boys are more likely to be born preterm: a novel mechanism for maintaining the fetal membranes in pregnancy
Kirsty G Pringle

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The rates of spontaneous preterm labour and premature rupture of membranes are higher in women with male infants. The causes of this sex difference are unknown but the intrauterine tissues have been suggested to regulate fetal growth and survival in a sex-specific manner. We have demonstrated that decidua from women carrying a female baby produce higher levels of prorenin during pregnancy. We are focussing on the role(s) of the decidual renin-angiotensin system (RAS) in maintaining the fetal membranes and how sex-specific alterations in decidual RAS expression contribute to the increased risk of preterm birth in male babies. The renin-angiotensin system is known to stimulate fibrosis in organs like the heart and kidney. We have demonstrated that before labour, there are lower levels of expression of prorenin in decidua from women carrying male babies and a decreased ability of decidual explants from these ‘male’ pregnancies to produce prorenin. Since we have identified fetal trophoblasts in late gestation decidua, we propose that this sex-specific difference in prorenin secretion is regulated by paracrine secretions from these placental cells and begins early in pregnancy. This may explain the increased susceptibility of the male fetus to preterm birth. In addition, we have demonstrated that female amnion shows higher expression of the (P)RR, which we propose is necessary for activation of pro-fibrotic pathways within the amnion since there is a strong correlation between (P)RR and the pro-fibrotic factor TGF-β. Our findings demonstrate that there are strong interactions between prorenin, (P)RR and TGF-β and that this system has a greater capacity in female amnion to stimulate fibrosis. More research is needed to investigate whether this pathway and other pro-fibrotic molecules (collagen, PAI-1 and fibronectin) play a functional role in regulating membrane integrity and if this pathway is dysregulated in women with preterm premature rupture of membranes.

Uncovering new roles of chromatin: control of gene activation and splicing by testis-specific histone variant, mH2A.B/H2A.Lap1.
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Every cell within an organism contains the same genome, yet each specific cell type only allows expression of a subset of genes that defines its function. To achieve this, very precise DNA handling mechanisms must be in place. One such mechanism is epigenetic regulation of genome. Epigenetic regulation functions on multiple levels: from gene expression to DNA repair to flawlessness of chromatin between daughter cells during mitosis. However, one of the most dramatic examples of epigenetic regulation is during spermiogenesis. Indeed, spermatozoa are the only cell type that is destined to leave the parental organism and ultimately, outlive it. To achieve this, haploid spermatids undergo sequential differentiation steps, all tightly controlled by epigenetic machinery, so that, acrosome and flagellum are formed, nucleus is markedly condensed and cytoplasm is lost. To begin this transformation, a robust gene activation program must be switched on by round spermatids at the onset of spermatogenesis.

Here, I will discuss a new role for H2A histone variants in regulation of gene activation and mRNA splicing in round spermatids. Our findings show that testis-specific histone variant, mH2A.B/H2A.Lap1, is associated with transcription start sites of actively transcribed genes, co-localises and interacts with RNA Pol II complex subunits and, unexpectedly, interacts with a number of RNA-binding proteins that control pre-mRNA splicing. Interestingly, our immunofluorescence staining and subcellular fractionation of round spermatids revealed that a major fraction of mH2A.B, while still being bound to chromatin, localized within splicing speckles with actively transcribing RNA Pol II complex and RNA processing factors, showing that mH2A.B associates not only with DNA but also with RNA, specifically at the sites of splicing. Finally, we showed that N-terminus of mH2A.B directly binds RNA. This is a first report, to our knowledge, of direct interaction between a histone N-terminal tail and RNA.

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**High circulating fetal progesterone elevates fetal free cortisol levels through cortisol displacement from corticosteroid-binding globulin**

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Background: Glucocorticoids are essential for fetal development and organ maturation, and therefore normal transition to extra-uterine life. This process is facilitated by a natural increase in maternal cortisol as pregnancy progresses. Cortisol bioavailability and local tissue delivery is regulated by corticosteroid binding globulin (CBG). Progesterone also binds to CBG, however with over a 3 fold lower affinity than cortisol. In order to understand the regulation of cortisol bioavailability in utero, we assessed gestational changes in free and total cortisol, CBG (both high and low affinity forms; haCBG and laCBG) and progesterone in cord blood from preterm and term deliveries.

Methods: Cord blood was collected from preterm (n=141) and term (n=176) neonates at the Women’s and Children’s Hospital, Adelaide. Ha/la CBG levels were assessed by an in-house ELISA. Total cortisol and progesterone were measured by electro-chemiluminescence immunoassay and ELISA, respectively. Free cortisol fraction was determined using a temperature-controlled ultrafiltration/ligand-binding method. Clinical data were extracted from medical records.

Results: Cord blood total and free cortisol, and the proportion of haCBG, increased significantly across pregnancy (p<0.05). Cord blood progesterone levels were over 100-fold those in women in the luteal phase (mean 7476nM/L, IQR 4184-9697nM/L), and did not significantly rise over pregnancy. Free cortisol fractions were elevated approximately 3-fold in gestation. While the progesterone to CBG ratio did not change over gestation, it was correlated with free cortisol concentrations at each gestational time point (r=0.155, p=0.03).

Conclusion: A high free cortisol fraction in utero may allow a fetal-specific, cortisol tissue distribution necessary for development. This is not driven through altered CBG ha/la levels. High progesterone levels found in cord blood suggest a ‘progesterone switch’ in CBG function in utero, resulting in displacement of cortisol from CBG. This results in predominant free cortisol in the neonatal circulation, with potential important physiological implications for neonatal transition.

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**High maternal corticosterone levels during pregnancy programs sex-specific alterations in adrenal morphology and function in adult offspring of mice.**

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Stress during pregnancy programs long-term health outcomes. The hypothalamic-pituitary-adrenal-(HPA) axis plays a central role in regulating disease outcomes, but the impact of increased endogenous glucocorticoid concentrations on the structure and function of offspring adrenal glands has not been thoroughly investigated. This study aimed to identify the effects of short-term, prenatal corticosterone (Cort) exposure in the mouse on offspring adrenal gland morphology and steroid hormone concentrations.

Cort was administered to C57Bl/6 mice via osmotic minipump (33mg/kg/h) for 60h from E12.5. Adrenals and plasma were collected from offspring at PN30 (adolescent), 6 months (adult) and 12 months (aged). Adrenals were fixed and processed for histological/analysis or frozen for qPCR analysis of steroidoigenic enzymes (Cyp11a1, Cyp21a1, Cyp11b1, Hsd11b2, Cyp11b2), cholesterol transport protein (Star) and ACTH receptor (Mc2r). Plasma Cort was measured by RIA and aldosterone by ELISA. Adrenal volumes were determined by stereological analysis using the Cavalieri method.
Prenatal Cort had no effect on adrenal parameters measured in females at any age. In males, adrenal weight was unaffected at PN30 but increased at 6 months (44%). Plasma Cort levels were similar between Cort and Unr animals at PN30 but Cort (71%) and aldosterone (44%) were both increased in 6 month-old male offspring. Adrenal volume was reduced in Cort-exposed male offspring at PN30, particularly in the zona fasciculata (36%) which contains the glucocorticoid-producing cells, but was increased by 6 months (52%). Interestingly, Mc2r was up-regulated (1.2-fold) at PN30 and Cyp11a1 was down-regulated (1.4-fold) at 6 months in Cort-exposed male offspring. At 12 months, Cort-exposed male mice showed enhanced age-induced plaque formation and fibrosis.

Prenatal Cort results in age-dependent changes to adrenal size, volume and steroidogenic gene expression in male offspring while females appeared unaffected. These findings suggest a role for the HPA in the etiology of sex-specific programming of disease.

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Nutrition, growth and developmental rate affect the timing of mammalian growth axis maturation

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Maturation of the mammalian growth axis occurs when the production of key growth factors in the liver, namely IGF-I, is responsive to circulating growth hormone (GH) via its interaction with hepatic GH-receptors. While this process occurs around the time of birth in some mammals such as sheep it occurs well after birth in others including humans, mice and marsupials. To determine if nutrition, which influences growth and developmental rate, influences the timing of growth axis maturation in mammals we organized day 60 post-partum tammar wallaby pouch young into slow, normal and fast growth groups and measured the expression and circulating concentrations of key genes and hormones including GH, IGF-I/II, GHR, IGFBP3 and IGFALS at 120 and 150 days post-partum. Slow young included those of primiparous mothers in their first reproductive year (n=7; maternal weight: 3.0 ± 0.4 kg), while normal young were those of multiparous females (n=16; maternal weight: 5.2 ± 0.5 kg). Fast young were fostered at day 60 post-partum to mothers at day 120 of lactation that produce a higher volume, energy dense milk, accelerating their growth and development (n=12; maternal weight: 5.1 ± 0.2 kg). Growth, development and maturation of GH/IGF-I axis components occurred earlier in fast growing young, which had significantly increased hepatic GHR, IGF1 and IGFALS expression, plasma IGF-I concentrations, and significantly decreased plasma GH concentrations compared to age-matched young in the normal and slow groups (p < 0.05, respectively). These data support the hypothesis that the timing of growth axis maturation depends largely on the growth rate and maturity of the young, which can be altered by changing their nutritional status.

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Restricted placental growth does not reduce spontaneous activity in the adolescent or young adult sheep.

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Intrauterine growth restriction (IUGR) increases the risk of metabolic diseases including type 2 diabetes (T2D) in adult life. Increased lifetime or childhood exercise or activity correlates with lower T2D risk (1,2), such that decreased activity in IUGR individuals might explain their increased risk of T2D. Retrospective human data suggests that IUGR decreases activity in adulthood (3,4), but such studies may be confounded by differences in the postnatal environment. Impaired placental function is a major cause of human IUGR in developed countries, and restricted placental function and hence fetal growth (PR) in sheep impairs postnatal glucose tolerance, insulin secretion and insulin sensitivity. We hypothesised that PR would decrease spontaneous activity in adolescent and adult sheep. Spontaneous activity in 14 control (CON: 5 M, 9 F) and 19 PR sheep (9 M, 10 F) was recorded as distance travelled during two 18-hour trials per sheep in adolescence (204 ± 1 d old) and young adulthood (294 ± 1 d old) using Garmin Forerunner 910XT GPS devices. Ewes and rams were housed in adjacent paddocks. PR reduced birth weight by ~12% in this cohort (P=0.015). In adolescents, total distance travelled during the trials did not differ between CON and PR sheep (P>0.6), was 12% greater in females than males (P=0.045), and correlated negatively with maximum daily temperature (P=0.041). In young adults, activity was greater in PR than CON (P=0.004) and did not differ between sexes (P>0.2). At both ages, distance travelled varied with time (each P<0.001), peaking during the evening and morning. These diurnal patterns of activity are consistent with previous studies in sheep (5). Our data suggests that PR does not reduce activity in adolescent or young adult sheep, and we are currently assessing whether greater levels of early life exercise predict better adult metabolic outcomes in sheep, as in humans.

Bisphenol A and childhood overweight and obesity: is there a link?

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Background: Experimental models suggest that exposure to bisphenol A (BPA) in early life promotes excess adiposity, but it is unclear whether BPA exposure in human populations plays a role in childhood obesity. The objective of this study was to investigate the relationship between childhood exposure to BPA and excess body weight from human epidemiological studies.

Methods: Eligible studies were identified by systematic searches of Pubmed, Embase, Cochrane and Toxline databases, until 15th May 2015. There were no language restrictions and reference lists of relevant publications were also searched. Longitudinal cohort, cross-sectional and case-control studies were included if they reported urinary BPA concentrations in children. The primary outcome measures were age-and-sex-adjusted BMI percentile of ≥ 85th percentile for overweight and ≥ 95th percentile for obesity. High vs low dose analyses were used to calculate the pooled ORs, by comparing the odds of being overweight and obese for children in the highest vs lowest BPA exposure categories for each study. Linear dose response analyses were then preformed for exposure in school aged children using generalised least square trend estimation.

Results: Seven studies published between 2009 and 2014 were included, involving 4897 children worldwide. For children in the highest BPA exposure category the pooled OR for child overweight was 1.38 (95% CI 1.16 to 1.63, P < 0.0001) and child obesity was 1.56 (95% CI 1.26 to 1.92, P < 0.0001); compared to those with the lowest levels of exposure. Dose-response analysis found that for each 1 µg/L increase in child urinary BPA concentration, the pooled OR for child obesity increased by 4% (OR=1.04; 95% CI 1.01 to 1.07, p < 0.003).
Conclusion: BPA exposure is associated with a significant increased odds of overweight and obesity, providing a compelling argument that BPA promotes excess body weight and contributes to obesity in human children.

Aging at the Start of Life.

Roger Smith

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Aging is the pathophysiological process that causes the likelihood of death to increase with advancing age. It can be likened to the wearing out of any machine with time and use. Biologically aging is a consequence of damage to the organism exceeding repair. Peter Medawar posited that this aging process is tolerated after reproduction has occurred, as adverse events after reproduction have little effect on the retention of the causal genes within the gene pool. This effect is called Medawar's Shadow, the Shadow is the period of time the organism lives after reproduction. This effect explains why damaging genes, such as those for Huntington's chorea remain in the genome, as the effects of Huntington's occur after reproduction, it is even thought that some conditions that have adverse effects later in life might increase reproductive success at an earlier stage of life.

The placenta is an unusual organ that is only required for 9 months. It is therefore possible to examine human aging related pathways in a tractable time frame by studying the placenta. Almost all pregnancies have delivered by 40 weeks. Thus the reproductive functions of most placentas have been completed by 40 weeks. We have compared aging related pathways in placentas delivered at 38 weeks with those delivered after 40 weeks. We have identified major changes in the function of the mTOR pathways and in protein synthesis and degradation that occur in the final weeks of pregnancy. It is likely that these aging related changes lead to the rapid increase in stillbirth risks that occur in late gestation.

Ageing, appetite and body composition

Ian M Chapman

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Normal ageing is associated with changes in eating behaviour and body composition. The body weight (or BMI) associated with greatest life expectancy increases with age, and is ~27-28 kg/m2 in people > 70 years, compared to < 25 kg/m2 in younger adults, yet on average older people lose body weight after about age 65 years. This weight loss is often involuntary, and related to the physiological decline in appetite that accompanies normal ageing (“anorexia of ageing”). The causes of this appetite decline are multiple, but include reduced sense of smell and taste, slowing of gastric emptying and possibly hormonal changes such as increased activity of the satiating hormone cholecystokinin. When pathological factors, such as depression, inflammation, malignancy and social isolation, are superimposed, weight loss can become excessive and lead to pathological under-nutrition. The association between weight loss (particularly if > 5% and involuntary) and/or low body weight (BMI < 22 kg/m2) and adverse outcomes, has been demonstrated clearly in older people. This is related, at least in part, to the body composition changes accompanying ageing. As people age they lose skeletal muscle, and when they lose weight the tissue lost is disproportionately skeletal muscle. In contrast, ageing is associated with in overall increase in fat stores and a redistribution of these stores to be intrahepatic, intramuscular and intra-abdominal (cf subcutaneous) deposits. When excessive the loss of skeletal muscle leads to sarcopenia and frailty, which are particularly associated with adverse outcomes, including reduced quality of life and increased morbidity, need to move into higher level accommodation, and mortality. Approaches to under-nutrition and sarcopenia shown to be of benefit in older people include identification and correction of underlying causes, the use of protein-enriched nutritional supplements and exercise programs (particularly resistance exercise), but not, to date, the use of anabolic hormones.
Evidence continues to emerge that alterations in telomeres are associated with a wide range of pathologies. Telomeres cap the ends of chromosomes and contribute to genomic stability. They also shorten slightly every time a somatic cell divides, which sets an upper limit on cellular proliferative capacity. The great majority of cancers evade this normal limit on cellular proliferation by acquiring the capacity to counteract normal telomere shortening via increased activity of a telomere lengthening mechanism – either the telomerase reverse transcriptase, or a recombination-dependent telomere copying process. In contrast, there is a growing list of mutated genes causing excessive telomere shortening and therefore premature proliferative failure in various organ systems, with protean clinical manifestations that are collectively known as “short telomere syndromes” (or “telomeropathies”). Prominent clinical manifestations include bone marrow failure, mucocutaneous abnormalities and pulmonary fibrosis, but many organs and tissues may be affected. Paradoxically, there is a high risk of cancer, presumably because of genomic instability resulting from failure of the telomeric capping function. Endocrinologists may be consulted about short stature or early-onset osteoporosis which are relatively common or, less commonly, hypogonadism, or for management of the side effects of androgen treatment for bone marrow failure, of corticosteroid treatment of graft-versus-host-disease, or of cancer treatment. Between the two extremes of cancer – where telomere shortening is completely prevented – and the excessive telomere shortening characteristic of the short telomere syndromes, more moderate changes in telomere length appear to be risk factors for a range of common diseases. A number of lifestyle and metabolic risk factors, including obesity, physical inactivity, and insulin resistance, have been associated with shorter telomeres. Cross-sectional studies have shown an association between shorter telomeres and type 2 diabetes, but the causal relationship is not clear. Possible explanations include that (i) short telomeres are a biomarker of known risk factors, (ii) short telomeres contribute directly to aetiology of type 2 diabetes, perhaps through proliferative failure of islet cells, and/or (iii) the metabolic disturbances characteristic of this condition cause telomere shortening. However, there is some evidence that short telomeres are an independent risk factor for type 2 diabetes.

Osteoporosis may be present

- Treatment of BMF with androgens – requires periodic endocrine evaluation
- Patients receiving androgen therapy should be monitored 2732 regularly by an endocrinologist for androgen-associated side effects impacting growth, bone age (early fusion of epiphyses), 2734 gonadal function, and lipid profile in case there is a need for 2735 intervention. Persistently low HDL and high LDL levels may be 2736 2737 2738 2739 2740 2741 2742 2743 2744 2745 of concern for future cardiovascular risk in patients on long-term androgen therapy, but usually return to baseline values within 3–6 months after discontinuing the androgen treatment. Thyroid function is not affected by androgen therapy, but thyroid binding globulin level has been found to be reduced in patients using oxymetholone.

Despite preventive measures, patients may still develop GVHD, ranging in severity from limited skin involvement to 3385 life-threatening multi-organ failure. Corticosteroids such as methylprednisolone are first-line therapy for GVHD, and adequate control may require long-term immunosuppression

DC patients should undergo regular, comprehensive multi-3487 disciplinary evaluations with appropriate targeted testing in the 3488 years following HCT. Late effects of alkylating agents and radiation include malignancy, fertility problems, and endocrine defects, which are known DC-associated complications. Chronic GVHD and prolonged use of corticosteroids or other immunosuppressive therapies may exacerbate bone disease and magnify risk of malignancy in DC.

Overlap between manifestations 3560

- of DC and HCT late effects
- Endocrine
- Skeletal defects, short stature, Thyroid defects, growth hormone deficiency, fertility problems, hypogonadism, hypogonadism, metabolic syndrome

It appears that endocrine hormone deficiencies, such as 4976 hypothyroidism, growth hormone deficiency (GHD), 4977 hypogonadism, diabetes, or short stature, are not 4978 common in patients with DC. However, abnormalities related to the skeleton 4979 are seen with higher incidence compared to the general 4980 population.

Reported endocrine and skeletal abnormalities in DC 4991

**Features**

<table>
<thead>
<tr>
<th>Short stature</th>
<th>20% DC registry in United Kingdom</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypogonadism/undescended testes</td>
<td>6%</td>
</tr>
<tr>
<td>Osteoporosis/avascular necrosis/scoliosis</td>
<td>5%</td>
</tr>
<tr>
<td>Osteopenia/vascular necrosis</td>
<td>10% Literature review</td>
</tr>
<tr>
<td>Growth hormone deficiency</td>
<td>1 patient Case report</td>
</tr>
</tbody>
</table>

Bony abnormalities, including AVN of the hips and shoulders, osteopenia and osteoporosis, and scoliosis were reported in 4995 approximately 5% of patients with DC1, but may be more frequent than reported to date (unpublished data – NCI cohort). Many patients with DC (~75%) also have dental abnormalities 4998 such as shortened roots or taurodontism.

**Growth and growth hormone 5070**

Short stature is reported in 12% of cases in the literature and 5071 in approximately 20% of patients in the UK DC registry. 5072 In contrast, the NCI cohort notes that short 5073 stature is very rare in individuals with DC, perhaps being more 5074.

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Roger Reddel

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**Table:**

- **Source:**
  - DC registry in United Kingdom
  - Literature review
  - Case report

**Growth and growth hormone 5070**

Short stature is reported in 12% of cases in the literature and 5071 in approximately 20% of patients in the UK DC registry. 5072 In contrast, the NCI cohort notes that short 5073 stature is very rare in individuals with DC, perhaps being more 5074. **Chapter 5: Genetic Counseling**
common in very severely affected patients. While the precise 5075 mechanism is unknown, their short stature does not appear to be 5076 related to growth hormone deficiency, and growth hormone 5077 therapy is not recommended unless the patient is proven to have 5078 this deficiency.

**Hypogonadism 5080**

A small number of severely affected males reported decreased 5081 sperm or testosterone production, or both, a condition known as 5082 hypogonadism. Animal models have demonstrated that at 5083 least one of the D mutations may lead to testicular atrophy in 5084 males and decreased fertility in both males and females, but 5085 this has not been duplicated in human studies.18,19

**Origins of Primary Aldosteronism**

William Rainey

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Since the evolution of species from oceans to land, the adrenal steroid hormone, aldosterone, has played a critical role in the maintenance of fluid and sodium homeostasis. Aldosterone production is normally tightly controlled by circulating potassium (K⁺) and the renin-angiotensin system (RAS). The adrenal’s capacity to produce aldosterone relies heavily on the expression of a single enzyme, aldosterone synthase (CYP11B2). This enzyme carries out the final reactions in the synthesis of aldosterone and is expressed almost solely in the adrenal zona glomerulosa. Our research has demonstrated that angiotensin II and K⁺ cell signaling pathways converge on calcium-dependent pathways that increase CYP11B2 expression and aldosterone production. This process involves activation of calmodulin (CaM), CaM kinases, and select transcription factors, ending with increased CYP11B2 expression and aldosterone production. From a disease standpoint, aldosterone excess is the most common of all adrenal disorders. The prevalence of autonomous aldosterone excess (primary aldosteronism, PA) occurs in approximately 1 in 30 adults, accounting for up to 10% of hypertension and up to 20% of resistant hypertension cases. The major causes of PA are adrenal aldosterone-producing adenomas (APA) and adrenal idiopathic hyperaldosteronism (IHA). In both conditions, CYP11B2 expression and aldosterone synthesis occur in a renin-independent manner. Despite the common occurrence of PA, little is known about its cellular origins. We have recently identified clusters of cells within normal adrenals that have inappropriate expression of CYP11B2. Through the use of genomic approaches, we have demonstrated that these cell clusters have mutations in genes that disrupt normal cellular calcium homeostasis, leading to renin-independent aldosterone production. These findings support the concept that adrenal cell calcium homeostasis is an important regulator of both normal and pathologic production of steroids. In addition, somatic gene mutations that alter intracellular calcium homeostasis and cause renin-independent aldosterone production are quite common, even in normal adrenals. Studies to define the mechanisms causing these dysplastic cells to expand in number and cause PA are underway.

**Fetal programming in 2yo calving heifers: Effects of maternal peri-conception and first trimester protein supplementation on progeny feedlot performance, appetite and carcass characteristics**

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Fetal developmental programming is a well established concept. Maternal nutrition has been observed to influence offspring development in livestock and other animal species. This experiment evaluated the effects of dietary restriction in yearling heifers during the peri-conception period and first trimester upon progeny growth, physiology and reproduction. The feedlot performance, appetite and carcass characteristics of the offspring are presented here.

*Bos Indicus* cross heifers were individually fed high (14% crude protein(CP)) or low (7%CP) diet for 60 days prior to conception. At 23 days post-conception, each high(HPERI) or low(LPERI) group was again split into high (HPPOST) or low (LPPOST) diet groups in a 2x2 factorial design. From the end of the first trimester (98dp) heifers (n=64) were fed to meet nutritional requirements until term. The singleton entire male progeny (n=39) were fed to a standard industry endpoint before slaughter at 18-months.

Liveweight and feed conversion ratio between bulls did not differ due to maternal diet (Two-way ANOVA; P>0.10). Feed intake varied with a significant interaction (P<0.05) between maternal PERI and POST diet such that progeny of dams that had a change in diet (LH and HL) had 9% higher daily feed intake than those whose dams received low diet throughout the PERI- and POST-conception period (LL). A similar pattern was apparent in feedlot growth-rate and net feed intake but the differences were not significant (P>0.10). Carcass weight was similar, however dressing%, yield and eye muscle area (88.0±2.61vs81.1±1.8cm²) were higher (P<0.05) for HPPost bulls who also tended to be leaner (15.7±1.2vs18.7±0.6mm; P=0.6).

Combined, these results suggest that the diet perturbation in early gestation has altered the pattern of development of both the appetite regulatory system and fetal skeletal muscle leading to persistent post-natal effects.

Acknowledgements: We are indebted to S. Kidman and Co., Ridley Agriproucts and ARC for funding this research.
Penicillamine attenuates the agglutination of ram sperm in capacitating media

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Robust in vitro capacitation protocols are necessary to assess the physiological mechanisms underlying the preparation of sperm for fertilisation. Here we describe the unreported phenomenon of head to head agglutination of ram sperm following extension in the capacitating media, Tyrodes plus albumin, lactate and pyruvate (TALP). Agglutination is immediate, specific, persistent and is not associated with a loss of motility or viability. Agglutination can be prevented or reversed by penicillamine (PEN). A dose of 250µM PEN reduced the percentage of motile, agglutinated sperm from 77 ±3% to 3±1%. Reversion back to the agglutinated state can be achieved by adding 5µM copper but other heavy metals including cobalt, iron, manganese were not as effective.

PEN can act as a chelator of heavy metals, an antioxidant and a reducing agent. To investigate PEN’s mechanism of action we compared it to the broad spectrum chelator ethylenediaminetetraacetic acid (EDTA; 1mM) and the copper specific chelator (bathocuproinedisulfonic acid (BCS: 1mM). BCS and EDTA significantly increased the percentage of motile, non-agglutinated sperm compared to the control but were significantly lower than PEN (1mM). The antioxidants superoxide dismutase (SOD; 800 IU mL⁻¹) and ascorbic acid (1 mg mL⁻¹) showed similar low level inhibition of agglutination compared to PEN (1mM) and catalase (150 IU mL⁻¹) had no effect. At 0 hrs only the reducing agents cysteine (1mM) and DL-dithiothreitol (1mM) displayed similar levels of non-agglutinated sperm compared to PEN (1mM) but were less effective after 3hrs of incubation (37°C). Together these results indicate that PEN is an effective agent to reduce agglutination of ram sperm in capacitating media. It may be acting upon sulphydryl bonds of a sperm membrane protein that binds copper.

Y2 Receptor ligands act within the median eminence to regulate Gonadotropin Releasing Hormone (GnRH) Secretion

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Neuropeptide Y (NPY) is stimulates food intake and inhibits reproduction. In the sheep, we have shown that the suppressive effect of NPY on reproduction axis is via the Y2 receptor (Y2R) (1). The present study aimed to determine whether GnRH secretion is inhibited, via the Y2R at the level of the GnRH neurosecretory terminals in the median eminence of the sheep. Firstly, dual-labelling immunohistochemistry for NPY and GnRH confirmed projections into the median eminence of the sheep brain. Secondly, to determine whether a Y2 selective ligand can inhibit GnRH secretion we injected NPY 13.36 µg into the median eminence of conscious ovariectomised (OVX) ewes (n=5). Injections of 1 nanomole NPY 13.36 µg were in 50 nanolitres 0.9% saline vehicle. Blood samples were taken from the jugular vein at 10 min intervals for 90 min prior to and following the injections of the agonist or vehicle, to measure plasma luteinizing hormone (LH) levels. This tested whether the agonist could act directly on GnRH neurosecretory terminals. The agonist caused cessation of pulsatile LH secretion and reduced mean (±SEM) LH levels from 1.7±0.23 to 1.1±0.18 (P<0.05), with no effect of vehicle. Dual label immunohistochemistry in the median eminence showed localization of the Y2R to GnRH neurosecretory terminals. In situ hybridization for the Y2R showed labelling of cells in the preoptic area of the ovine brain and studies in progress will determine the cellular localization of these receptors. These data provide strong evidence that GnRH secretion maybe inhibited by NPY, originating from the arcuate nucleus of the brain. Because the neurosecretory (external) zone of the median eminence is outside the blood-brain barrier, there is also the possibility that circulating peptide YY (Y2R ligand) from the gut may negatively regulate GnRH secretion at this level.

Reference


Regulation of Fibrillin Genes During Development of the Bovine and Human Ovary

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Fibrillins are extracellular matrix proteins that play important roles in regulating TGFβ activity. TGFβ stimulates fibroblasts, present in ovarian stromal compartments, to proliferate and synthesise collagen. Stromal expansion in the developing fetal ovary is crucial to ensure correct ovarian structural organisation and function. Expression of FBN2 has been associated with

Helen F Irving

Nicole A Bastian

Regulation of Fibrillin Genes During Development of the Bovine and Human Ovary

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Fibrillins are extracellular matrix proteins that play important roles in regulating TGFβ activity. TGFβ stimulates fibroblasts, present in ovarian stromal compartments, to proliferate and synthesise collagen. Stromal expansion in the developing fetal ovary is crucial to ensure correct ovarian structural organisation and function. Expression of FBN2 has been associated with
PCOS in women (JCEM, 2006. 91: 4112-7). However, it is not expressed in the adult ovary, only in the stroma of the fetal ovary and only during the first trimester (FASEB J, 2011. 25: 2256-65). FBN1 is expressed at constant levels and at higher levels in the adult ovary (FASEB J, 2011. 25: 2256-65). We examined the expression of FBN1-3 in bovine and human fetal ovaries in vitro. Following 24 h of culture of collagenase-dispersed bovine fetal fibroblasts from the first trimester, the expression of FBN1 significantly increased but FBN2 and FBN3 significantly decreased. When undispersed bovine fetal ovarian tissue was cultured, FBN1 expression remained unchanged, however expression of both FBN2 and FBN3 declined. TGFβ-1 decreased FBN1 and FBN2 expression only in bovine first trimester fibroblasts, but did not affect FBN3 expression. Additionally, human fetal (9-17 weeks gestational age) ovarian somatic cells were cultured over a number of passages. FBN1 and FBN2 expression increased whereas FBN3 expression drastically decreased. The cultured ovarian somatic cells were then treated with TGFβ-1 or SB431542. Interestingly, TGFβ-1 and SB431542 differentially regulated FBN1 and FBN2 expression in these somatic cells. Overall these results suggest that in vitro ovarian fibroblasts from fetal ovaries switch off FBN2 and switch on FBN1 as occurs in vivo during development. Additionally the regulation of these genes changes over gestation. The mechanisms responsible are not known at this time. However, we have established a model system for the study of developmental changes in the ovarian somatic compartment.


Detection of aggregated transthyretin in placental extracellular vesicles: importance for preeclampsia

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Preeclampsia is a hypertensive disease affecting 3-5% of otherwise healthy pregnancies. The pathogenesis of preeclampsia is unclear but it is known that placent al toxin(s) trigger the disease. Transthyretin, a carrier protein for thyroxine/retinol, which is aggregated and cytotoxic in preeclamptic serum may be one such toxin. We undertook this study to investigate whether transthyretin production is altered in preeclamptic placentae and whether this potential “toxin” is carried into the maternal circulation by placental extracellular vesicles.

RNA and protein levels of transthyretin in preeclamptic and control placentae were investigated by qRT-PCR, immunohistochemistry and Western blotting. Macro-, micro- and nano- vesicles were harvested from cultured placentae by differential centrifugation (2000g, 20000g, 100000g). Total aggregated protein in each vesicle fraction was quantified by Proteostat® assay and the levels of monomeric/aggregated transthyretin were determined by Western blotting.

The level of transthyretin protein (n=7; p<0.011), but not transthyretin mRNA (n=8), was increased in preeclamptic placentae. Transthyretin was detected in micro- and nano-vesicles, but not macro-vesicles, from first trimester (n=5) and term placentae (n=5). The level of monomeric and aggregated transthyretin was significantly increased in nano-vesicles, but not micro-vesicles, from preeclamptic placentae compared to control placentae (n=8; p=0.0127). Nano-vesicles from preeclamptic placentae also contained higher total levels of protein aggregation (n=7; p=0.0136).

We have shown that the placenta actively “secretes” transthyretin into the maternal circulation throughout gestation via placental micro- and nano- vesicles. That the levels of transthyretin mRNA in preeclamptic placentae was unchanged, while the protein level in both the placenta and nano-vesicles was increased, suggests that post-transcriptional modifications to transthyretin are affected in preeclampsia, leading to increased transthyretin aggregation, and transport into the maternal blood via nano-vesicles. The presence of aggregated transthyretin in nano-vesicles may alter the interaction between these vesicles and maternal cells, contributing to the clinical symptoms of preeclampsia.

Marginal zinc deficiency in mice during pregnancy and lactation reduces fetal growth and increases maternal blood pressure

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Placental research is increasingly focused on how the organ adapts to support adequate fetal growth in a potentially sub-optimal nutritional environment. However, the precise mechanisms by which specific micronutrients, such as zinc, contribute to pregnancy success remain unknown. Evidence suggests an association between maternal zinc deficiency and the development of placental-related pregnancy complications such as preeclampsia and intrauterine growth restriction. We used a dietary deficient mouse model to determine the effects of zinc deficiency on placental and fetal development and pregnancy
outcome. Seven week old C57Bl6 females were fed either a control (40 mg/kg zinc) or zinc deficient (10 mg/kg) diet (n=30 per group) for 6 weeks prior to mating and throughout pregnancy. A small subset of animals was selected to undergo radio-telemetry surgery to continuously measure blood pressure throughout pregnancy and lactation. The remaining females were mated and at day 18.5 of gestation, pregnant dams were sacrificed and placentas and fetuses weighed and collected for histological and molecular analyses. Average 24-hour mean arterial pressure was significantly elevated in the zinc deficient mice prior to pregnancy (p<0.01) and continued to increase throughout pregnancy and lactation (p<0.01 for both). Marginal zinc deficiency prior to and during pregnancy resulted in a 7% decrease in fetal weight (p<0.01) and pups were significantly smaller 3 days after birth until weaning (12% lighter and 33% lighter; respectively, both p<0.01). The decreased fetal weight was accompanied by a 10% reduction in placental weight at day 18.5 of gestation (p<0.01). However, structural analysis of the zinc deficient placentas revealed no significant differences in the mid-sagittal cross-sectional areas of the junctional and labyrinth zones despite a significant 12.5% reduction in labyrinth weight (p<0.05). These indicate potential compensatory mechanisms that render the zinc deficient placenta structurally similar but smaller than controls but remain insufficient to maintain fetal growth.

### Dietary-induced obesity suppresses expression of the nuclear receptor Reverba in placenta and fetal liver; implications for circadian and metabolic development

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4. Children's Nutrition Research Centre, School of Medicine, University of Queensland, Brisbane

Obesity during pregnancy causes adverse outcomes for both mother and fetus, but the specific mechanisms behind these complications remain unclear. Circadian variation is an important feature of normal metabolic function and undergoes marked changes across pregnancy. Consequently, obesity-induced disruptions to the circadian system may drive adverse outcomes in obese pregnancy. We previously reported that obesity suppresses clock gene expression in maternal liver and adipose tissue; this study investigated whether clock gene expression in fetal and placental tissues is also affected by maternal obesity.

Female Wistar rats were fed either chow (CON) or a cafeteria diet (CAF) for 8 weeks to induce obesity, then mated and maintained on the diets throughout gestation. Fetal liver and placental labyrinth zone (LZ) samples were collected at four-hourly time points across days 15 and 21 of gestation. Expression of clock genes was analysed by RT-qPCR. CAF animals exhibited a 58% increase in body fat compared to CON as measured by DEXA analysis (P<0.001). All clock genes were expressed in fetal liver and LZ but their expression patterns did not follow the characteristic circadian profiles observed in maternal tissues. CAF consumption suppressed peak expression of Reverba in both fetal liver and LZ (P<0.05), similar to observations in maternal tissues. Cosinor analysis showed phase changes in Rora and Per1 in fetal liver and Rora and Per3 in LZ (all P<0.05). Unlike maternal tissues, there were no dietary effects on expression of Bmal1, Cry2 and Per2 in LZ or fetal liver.

In summary, obesity-induced changes to clock gene expression extend to the fetal and placental compartments, although to a lesser extent than in maternal tissues. Reverba suppression may be particularly significant given its direct role in adipogenesis and lipid metabolism and could thus be a potential mechanism for programming effects in offspring of obese mothers.

### Prenatal exposure to the plasticizer bisphenol A (BPA) and adverse birth outcomes in human epidemiological studies

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Background: Incidence rates of pre-term birth have increased over the past 2-3 decades. It is possible that exposure to chemical contaminants during pregnancy may play a role. The objective of this study was to investigate the relationship between prenatal exposure to the plasticizer bisphenol A (BPA) and adverse birth outcomes from human epidemiological studies.

Methods: Eligible studies were identified by systematic searches of Pubmed, Embase, Cochrane and Toxline databases, until 15th May 2015. Longitudinal cohort, cross-sectional and case-control studies were included if they reported maternal serum or urine BPA concentration during pregnancy, as a marker of prenatal exposure. The primary outcome variables were (a) small for gestational age (≤ 10th percentile) and (b) preterm birth (≤ 37 weeks gestation). High vs low dose analyses were used calculate the pooled ORs, using the lowest BPA exposure category as the referent.

Nine studies published between 2010 and 2014 were included. Children with the highest prenatal levels of exposure to BPA had a pooled OR for SGA of 1.26 (95% CI 0.823 to 1.91, p = 0.284), compared to children with the lowest levels of exposure. However, on subgroup analysis, a significant association for SGA was seen in girls, (OR 2.65, 95% CI 1.24 to 5.73, p = 0.012); but not in boys. Children with the highest levels of prenatal BPA exposure had a pooled OR for pre-term birth of 1.37 (95% CI 0.96 to 1.84, p = 0.089), compared with children with the lowest levels of exposure.
Conclusion: A significant positive association was seen between exposure to BPA in pregnancy and SGA in girls, but not in boys. A positive trend which approached significance was also seen between prenatal BPA exposure and pre-term birth. Further investigation of the potential consequences widespread BPA exposure during pregnancy is worth further investigation.

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NAD⁺ availability is critical for meiotic maturation in mouse oocytes

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Sirtuins are NAD⁺-dependent deacetylases with a growing repertoire of functions, which are critically dependent upon NAD⁺ availability in somatic cells. Here we study the effects of altering NAD⁺ levels in oocytes using FK866, a highly specific inhibitor of the NAD⁺-generating enzyme, NAMPT, as well as a genetic approach involving transgenic mice overexpressing another NAD⁺-generating enzyme, NMNAT1 (NMNAT-Tg mice). We first investigated in vitro meiotic maturation, which stretches from germinal vesicle breakdown (GVBD) to polar body extrusion (PBE). GVBD was markedly inhibited in wild-type (WT) oocytes cultured in 30µM FK866 (P≤0.001). Significantly, GVBD rates for FK866-treated NMNAT-Tg oocytes (67%) were comparable to untreated controls and almost three-fold higher than FK866-treated WT littermates (23%; P=0.0034) reinforcing the importance of NAD⁺ homeostasis in oocytes and supporting that FK866-induced effects reflected NAD⁺ de-regulation. The impact on PBE was even more stark as PBE rates at 10h, 12h and 14h were drastically reduced by FK866 (P<0.0001), attaining maximal rates by 20h post-GVBD of only 16% versus 92% in controls. Although there was a clear trend towards improved PBE in FK866-treated NMNAT-Tg oocytes (two-fold higher), this was less marked than improvements observed for GVBD, suggesting that late stages of maturation are especially vulnerable to NAD⁺ de-regulation. Sirtuins are critical regulators of mitochondrial function and of glutathione production for antioxidant defences in somatic cells. We therefore examined mitochondrial activity, glutathione and levels of reactive oxygen species (ROS) during FK866 treatment using MitoTracker, MCB (monochlorobimane) and 2',7'-dichlorofluorescin diacetate (DCFDA), respectively. FK866-treated oocytes exhibited significantly reduced MitoTracker (P=0.008) and MCB (P<0.0001) fluorescence suggesting that reduced NAD⁺ availability led to reduced mitochondrial activity and less reducing power. ROS levels were also reduced (P<0.0001), likely secondary to lower mitochondrial activity. Collectively, these data show that reduced NAD⁺ availability severely impairs meiotic maturation producing defects consistent with deregulated sirtuin activity.
Plk1 is essential for establishing cortical actin polarity in mouse oocytes

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Female meiosis involves a highly asymmetrical division to form a large secondary oocyte and a small polar body. This process is important for retaining organelles and making a viable embryo. Polo-like kinase 1 (Plk1) is a serine/threonine kinase which is highly conserved from yeast to human and has been found to be a potent regulator of mitosis and meiosis involved in many cellular roles such as cytokinesis. Plk1 is known to regulate myosin via the activation of RhoA which leads to the contraction of the cleavage furrow in mitotic cells. In this study, it was observed that Plk1 inhibition in late metaphase I oocytes had disrupted cortical actin localisation. N-Wasp, which is an upstream protein in the actin polymerisation pathway, was not able to localise to the cortical region in these oocytes. However, when Plk1 was inhibited in MII eggs, no difference in oocyte localisation was observed which suggested regulatory differences between actin cap establishment and maintenance. In order to investigate this, MII eggs which had their actin caps depolymerised were treated with Plk1 inhibitor, and were then observed if they could then re-establish their polarised cortex. These eggs were found to have an absence of N-Wasp localisation with a significantly reduced actin presence. In conclusion, it is hypothesised that in meiosis I, Plk1 plays an important role in N-Wasp-mediated cortical actin establishment that complements the role of Plk1 in myosin activation and thereby provides a mechanism for coordinating events of cytokinesis.

Cumulin, an oocyte-secreted heterodimer of the transforming growth factor-β family, is a potent activator of granulosa cells and improves oocyte quality

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Growth differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP15) are oocyte-specific growth factors with central roles in mammalian reproduction, regulating species-specific fecundity, ovarian follicular somatic cell differentiation and oocyte quality. In the human, GDF9 is produced in a latent form, the mechanism of activation being an open question. Here, we produced a range of recombinant GDF9 and BMP15 variants, examined their in silico and physical interactions, and their effects on granulosa cells (GC) and oocytes. By generating a covalent BMP15 homodimer that cannot heterodimerize with GDF9, we found that the potent synergistic actions of GDF9 and BMP15 on GC can be attributed to the formation of a heterodimer, which we have termed cumulin. Modelling of cumulin revealed a dimerization interface identical to homodimeric GDF9 and BMP15, indicating likely formation of a stable complex. This was confirmed by generation of recombinant heterodimeric complexes of pro/mature domains (pro-cumulin) and covalent mature domains (cumulin). Both pro-cumulin and cumulin exhibited highly potent bioactivity on GC: activating both SMAD2/3 and SMAD1/5/8 signaling pathways in human granulosa COV434 cells, and promoting mouse GC proliferation (ED₅₀: 4ng/ml and 0.6ng/ml, respectively) and expression of a set of genes (Ptx3, Has2, Tnflap6, Ptg2s2) associated with oocyte-regulated GC differentiation towards the cumulus cell phenotype. In all cases cumulin was more potent than pro-cumulin, pro-GDF9, pro-BMP15 or the two combined on GC. However, on cumulus-oocyte complexes, pro-cumulin was more effective than all other growth factors at improving porcine oocyte quality using a low developmental competence model. Pro-cumulin increased subsequent blastocyst development 2.3-fold from 28% to 63%. Our results support a model of activation for human GDF9 dependant on cumulin formation through heterodimerization with BMP15. Oocyte-secreted cumulin is likely to be a central regulator of fertility in mono-ovular mammals.


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Heat Shock Proteins (HSPs) are highly conserved molecular chaperones, which increase during cytoprotective responses against cellular stress. Their main functions include protein folding and maintenance of cell viability; and some HSPs have been implicated in regulation of normal ovarian function and embryonic viability.

To understand how ovarian cells may respond to various stressors, we examined the expression and localisation of several key HSPs (namely, HSPA1A/B, HSPA5, HSP60, HSP90AA1, HSP90AB1, and HSP90B1) as well as transcription factor HSF1.
during oocyte maturation and ovulation in normal mouse ovarian follicles. Subsequent experiments will determine whether ovarian HSP expression is altered during metabolic stress, namely obesity, and its impact on ovarian function.

Ovaries were obtained from CBAF1 mice treated with PMSG to stimulate folliculogenesis, followed by ovulatory hCG for 0h, 8h, 11.5h or 13h. Paraffin sections were immuno-stained using antibodies against specific HSPs and peroxidase detection methods. Separately, cumulus-oocyte complexes and denuded oocytes were stained by immunofluorescence and visualised using confocal microscopy.

Every HSP was detected in granulosa cells as well as in the ovarian theca and showed dynamic regulation of expression in response to the LH surge. For instance, HSP90AB1 localised to granulosa and cumulus cells, with peak expression at the time of ovulation. Interestingly, the remaining studied HSPs showed dramatic changes in localization within the oocyte. Prior to the LH surge HSF1, HSPA5 and HSP60 localized within the Germinal Vesicle. Following the LH surge, HSPA1A/B and HSP90AA1 localized to the spindle; while HSP60 and HSP90B1 surrounded the meiotic spindle complex. In ovulated oocytes, only HSPA1A/B and HSP90B1 remained highly expressed respectively in the cytoplasm and the plasma membrane.

We show that the LH surge regulates HSF1 and HSPs expression in granulosa cells and triggers intracellular re-localization of these proteins in oocytes; likely reflective of their importance in cytoprotection and oocyte competence.

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**Integrated Approaches to Understanding Cancer Metabolism.**

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Altered cellular metabolism has emerged as a potentially important aspect in understanding tumour biology and may represent a novel therapeutic target. We have used an integrated approach to study metabolic reprogramming in pancreatic cancer - combining genomics, metabolomics, and phenotypic analysis on a unique cohort of patient-derived pancreatic cancer cell lines (PDCLs). Pancreatic cancer has a devastating prognosis, with five-year survival less than 5% and severely restricted treatment options. Although accumulation of mitochondrial mutations has been observed in various tumour types, including pancreatic cancer, to date there has been little effort to directly link these to metabolic phenotype. We identified somatic mutations in the mitochondrial genomes (mtDNA) of pancreatic tumours. Mutations were also identified in a targeted study of ~1000 nuclear genes important for mitochondrial function and metabolism. Phenotypic analysis of pancreatic PDCLs showed metabolic changes consistent with mitochondrial dysfunction. Metabolomic and radiolabelled substrate utilisation assays indicate induction of reductive glutamine metabolism and increased anabolic biosynthesis in pancreatic tumour cells. Hence, the heterogeneous genomic landscape of pancreatic tumours may converge on common metabolic phenotypes, with individual tumours adapting to increased anabolic demands via different genetic mechanisms. This model predicts a novel therapeutic strategy for pancreatic cancer through targeting key enzymes in reductive glutamine metabolism and fatty acid biosynthesis. More recently, we have been applying similar approaches to understanding the role of fatty acid metabolism in breast cancer.

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**Gestational diabetes mellitus and adverse pregnancy outcomes: the impact of different treatment targets at two major Australian maternity services.**

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**Objective**

There is insufficient evidence for treatment targets in Gestational Diabetes Mellitus (GDM). We aimed to explore the impact of different treatment targets on pregnancy outcomes.

**Methods**

An observational study was conducted of singleton births >20 weeks at Monash Health (MH) and Royal Women’s Hospital (RWH) from 2009-2013. Data (pregnancy details, maternal and neonatal outcomes) were obtained from each hospital’s pregnancy database. Outcomes for women with GDM at MH (n=2,891) and RWH (n=1,930) were compared [diagnosis: 2hr glucose ≥5.5mmol/L and/or fasting glucose ≥6.7mmol/L]. Descriptive statistics are presented. Multivariable regression analysis will be used to examine associations between GDM treatment and adverse outcomes.

**Results**

The prevalence of GDM and requirement for insulin at MH were 7.9% and 31%, and at RWH with stricter treatment targets were 6.3% and 47% respectively. Over half of women with GDM were overweight or obese. The rate of special care nursery admission (29.6% vs 17.0%) was higher at MH compared to RWH, but rates of induction of labour (30.6% vs 56.6%) and caesarean section (33.8% vs 39.5%) were lower (all p<0.001), partly reflecting hospital protocols. Babies of women with GDM were born later (mean gestation 39±2 vs 38±2wks, p<0.001) at MH compared to RWH, and had higher rates of respiratory distress (3.6% vs 1.2%, p<0.001), hypoglycaemia (9.9% vs 2.2%, p<0.001) and macrosomia (11.3% vs 9.5%, p=0.035), but...
lower rates of pre-term birth (8.5% vs 11.3%, p=0.001) and stillbirth (0.3% vs 0.7%, p=0.024). Rates of shoulder dystocia and jaundice were comparable.

Conclusions
Stricter treatment targets for GDM appear to reduce macrosomia, without increasing neonatal hypoglycaemia or special care nursery admission, but may be associated with more obstetric intervention.

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Background: Liquid Chromatography Tandem Mass Spectroscopy (LCMS) is increasingly replacing traditional immunoassays in endocrine testing by virtue of its specificity and lack of cross-reactivity. We investigated whether the newer, more specific urinary free cortisol (UFC) assays have comparable sensitivity in patients with new or relapsed Cushing’s disease.

Method: 69 consecutive UFC samples from two tertiary hospitals were analysed on LCMS (user defined cut off < 150nmol/day), Roche extracted immunoassay (manufacturer cut-off < 380 nmol/day), and Abbott unextracted immunoassay (manufacturer cut-off < 487 nmol/day, user defined cut-off < 280 nmol/day). Samples were classified as A) Cushing’s (new diagnosis on histology, or known Cushing’s/clinical features with at least one positive midnight salivary cortisol/dexamethasone suppression test), or B) Unlikely Cushing’s by two independent Endocrinologists.

Results: 31 UFCs were positive on at least one method, of which 12 were true positives using clinical classification as the gold standard. Roche had 26 positive UFC, followed by LCMS (n = 20) and Abbott (n = 13 user defined cut-off, n = 5 manufacturer cut-off). All UFCs from confirmed Cushing subjects were positive on the Roche, false positives included patients on prednisolone and acutely unwell patients. The more specific methods (LCMS, Abbott) missed 2 relapsed Cushing’s disease. Area under the curve was 0.99 for Roche (C.I: 0.98 – 1.0), 0.85 for Abbott (C.I: 0.71 – 0.99), and 0.80 for LCMS (C.I: 0.62 – 0.98). Using Abbott user defined cut-off had the strongest agreement with clinical classification (Kappa = 0.56), using manufacturer’s recommended cut-off missed 8 samples of Cushing’s disease.

Conclusions: Although Roche assay cross-reacts with prednisolone, it detected two patients with relapsed Cushing’s disease that were missed by more specific assays. ACTH increases upstream steroid metabolites which cross react with less specific UFC methods, this cross-reactivity might play a role in early detection.

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Differential associations of ferritin and 25-hydroxyvitamin D with fasting glucose and diabetes risk in community dwelling older men.

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Aims: High ferritin and low vitamin D concentrations are associated with an increased incidence and prevalence of diabetes mellitus but the strength and nature of the association in older adults remains unclear. We examined the roles of ferritin and 25-hydroxyvitamin D as independent predictors of glycaemia in older men.

Methods: Cross-sectional analysis of a population-based cohort study of 4,248 community dwelling older men aged 70-89 years in Perth, Western Australia. Plasma ferritin, 25-hydroxyvitamin D and glucose were assayed. Diabetes was ascertained from self-report, medication usage and fasting glucose concentrations. Multivariate analyses adjusted for age, smoking, BMI, waist/hip ratio, physical activity, hypertension, lipids, creatinine, CRP and medical comorbidity.

Results: There were 588 men with diabetes (13.9%). Ferritin was associated with fasting glucose in non-diabetic men (0.05 mmol/L per 1SD increase in ferritin, p=0.01). 25-hydroxyvitamin D was inversely associated with fasting glucose in non-diabetic men (-0.08 mmol/L per 1SD, p<0.001). Ferritin was not associated with prevalent diabetes (highest vs. lowest quartile; >225 vs
Neutral associations of testosterone, dihydrotestosterone and estradiol with fatal and non-fatal cardiovascular events, and mortality in men aged 17-97 years

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Context
Lower testosterone (T) levels have been associated with poorer health outcomes in older men, however, the relationship between T, dihydrotestosterone (DHT) and estradiol (E2) with cardiovascular disease (CVD) in younger men remains unclear.

Objectives
We assessed associations between endogenous sex hormones with mortality (all-cause and CVD) and CVD events, in community-dwelling men aged 17-97 years.

Participants and methods
T, DHT and E2 were assayed using liquid chromatography-mass spectrometry, and SHBG and LH using immunoassay, in 2,143 men from the 1994/5 Busselton Health Survey. Outcomes of death from any cause, CVD mortality and CVD events were recorded to December 2010 by data linkage. Cox proportional hazards regression was performed, adjusting systematically for age and other cardiovascular risk factors.

Results
Of the 1,804 men included in the analysis, there were 319 deaths, 141 CVD deaths, and 399 CVD events. Compared to the full cohort, men who died were older (70.4±11.0 vs 50.3±16.8 years), and had lower baseline T (12.0±4.4 vs 13.6±4.9 nmol/L) and DHT (1.65±0.64 vs 1.70±0.72 nmol/L), but higher E2 (64.0±32 vs 60.1±30.2 pmol/L). After adjustment for risk factors, T was not associated with mortality (adjusted HR=0.90, 95% CI 0.79-1.04; p=0.164 for every increase in 1 SD of T), CVD deaths (adjusted HR=1.04, 95% CI 0.84-1.29; p=0.708) or CVD events (adjusted HR=1.03, 95% CI 0.92-1.15, p=0.661). No associations were found for DHT, E2, SHBG or LH in the fully-adjusted analyses. Results were similar when the analysis was restricted to men free of CVD at baseline.

Conclusions
In men aged 17-97 years, T, DHT and E2 were not associated with mortality or CVD outcomes. This neutral association of hormones with CVD contrasts with prior studies in older men. Future interventional studies are warranted to assess the effects of T supplementation on risk of CVD events in men across ages.

Effects of Androgen Deprivation on the Biomechanical Function of the Lower-limb Muscles during Gait in Men

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Background and aims: Testosterone is important for maintaining muscle mass in ageing men, however it’s role in physical performance is unclear. We hypothesise that testosterone withdrawal causes differential deficits in leg muscle function. We aimed to assess effects of androgen deprivation therapy (ADT) for prostate cancer on functional mobility.

Methods: This prospective 12-month case-control study of men with localised prostate cancer included 29 cases (newly commencing ADT) and 24 controls (not receiving ADT), matched for age and radiotherapy. Video-based quantitative gait analyses (walking on level ground) was combined with computational musculoskeletal modelling to determine the following

Outcomes
- Lower limb muscle function
- Gait mechanics
- Biomechanical models

Conclusions: In older men, increased ferritin is associated with increased plasma fasting glucose concentrations; however it is not a predictor of overall diabetes risk. Higher 25-hydroxyvitamin D concentrations are independently associated with lower fasting glucose levels and reduced risk of diabetes. In older adults manipulation of plasma ferritin may not alter diabetes risk, whereas interventional studies are required to determine whether vitamin D supplementation reduce the incidence of diabetes as vitamin D levels are associated with other health indices.
Poor glycaemic control is associated with decreased survival in patients with diabetes following lung transplantation

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Diabetes Mellitus (DM) is common in lung transplant recipients and is a major risk factor for mortality. We undertook a prospective study to determine whether glycaemic control was associated with survival following lung transplantation (LTx). We collated all available fasting and random glucose and HbA1c results of the 195 consecutive patients who underwent LTx from 1/8/2010 – 1/8/2013. Patients were followed until 15/5/2015. Eighty-six patients with DM (pre-and post-LTx or new onset DM post-LTx) were included in analyses to avoid bias. Cox regression analyses were performed to determine the effect of glycaemic control on survival.

Patients had a mean of 1.3, 5.77 and 1.5 fasting glucose, random glucose and HbA1c tests in the first 3 months after LTx and a mean of 5.5, 21.4 and 5.6 tests throughout follow up. Of the 86 patients with DM, 28 (33%) died. Estimated mean survival in these patients was 3.6 (95% CI 3.3 – 4.4) years.

Mean glucose and HbA1c over the first 3 months following LTx were not associated with survival. However random glucose from 3 months until end of follow up was associated with reduced survival HR 1.31 (95% CI 1.13 – 1.52, p<0.001). Fasting glucose and HbA1c from 3 months until end of follow up were not associated with survival, although the sample size and relatively small number of tests performed may have influenced this result.

Our findings suggest that glycaemic control in the first 3 months following LTx is not associated with survival. However the 31% increase in mortality risk for each 1mM increase in mean random glucose over the longer term is significant. Tighter glycaemic control following lung transplantation may improve in survival.

The Mythology of Vitamin D Deficiency and Insufficiency

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Introduction Vitamin D deficiency is defined as a serum 25-hydroxy-vitamin D (25(OH)D) <30 nmol/L, a value presumed to signal the likelihood of osteomalacia or secondary hyperparathyroidism [1]. Vitamin D insufficiency is held to be present with values <75nmol/L for reasons that are less clear [2]. If correct, ~4% of individuals are ‘deficient’ and ~75% are ‘insufficient’ and in need of therapy [3]. We aimed to determine whether there is (i) a serum 25(OH)D that signals secondary hyperparathyroidism and, if so, by inference, an increased risk for bone disease, and (ii) another level above which serum parathyroid hormone (PTH) has reached a nadir and ceases to diminish.

Method Concentrations of 25(OH)D, PTH, calcium and creatinine measured in the serum of 10349 women and 3582 men were collected by Melbourne Pathology. We excluded persons <20 years, patients with hyper- or hypocalcaemia, chronic kidney disease and a 25(OH)D >180nmol/L.

Results Serum PTH correlated negatively with serum 25(OH)D with no evidence of a threshold 25(OH)D distinguishing persons with and without an elevated PTH; PTH was within the ‘normal’ range in over half (714/1416) of subjects with 25(OH)D ≤ 30 nmol/L. Nor was there a nadir or plateau; the higher the 25(OH)D, the lower the PTH (Fig 1). For both sexes, PTH was higher in ≥55 than <45 year olds (p<0.001) after adjusting for 25(OH)D, serum calcium and eGFR.
Conclusion

PTH is a continuous trait. There is no serum 25(OH)D threshold that sensitively discriminates persons with and without secondary hyperparathyroidism. Further work is underway examining the association between these measurements and bone microarchitectural deterioration. Given the limited evidence of bone disease based on histomorphometry or antifracture efficacy using vitamin D supplements in community dwellers [4,5], these data challenge the existence of an 'insufficiency' state.

Effect of Glucocorticoid on Brown Adipose Tissue Function in Humans – A Randomised Double-blind Placebo Controlled Cross-over Study

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Background: Glucocorticoid (GC) excess causes obesity. In animals, GC inhibits brown adipose tissue (BAT) function, leading to weight gain. The involvement of BAT in the development of obesity induced by GCs in humans is not known.

Aim: To investigate the effect of GC on BAT function in humans.

Method: In a randomised double-blind cross-over design, 10 healthy adults (6 men, 4 women; age mean±SEM, 28±6 year; BMI 25±3 kg/m²) underwent 1 week each of oral prednisolone (15mg/day) and placebo treatment with an intervening 2-week washout period. At the end of each treatment, under standardised cooling (19-20°C), BAT function was assessed by measuring (i) BAT activity on PET-CT scan after 75MBq of FDG (ii) supraclavicular (SCL) skin temperatures using infrared thermography (iii) energy production after a standardised meal using indirect calorimetry.

Results: Compared to placebo, SCL BAT activity (SUVmax 6.2±2.6 vs 3.7±1.4, P=0.08) and volume (44±26 vs 23±15cm³, P=0.09) were lower with prednisolone. During cooling, SCL skin temperature fell to a greater degree with prednisolone (-0.4±0.1vs -0.9±0.17°C, P=0.0005). Energy production was stimulated by the meal and the stimulation was significantly higher during prednisolone treatment (209±21 vs 292±34kcal/day, P=0.002). Postprandially, SCL skin temperature rose during placebo but fell during prednisolone treatment (+0.2±0.1 vs -0.3±0.1°C, P=0.009).

Summary: Prednisolone suppresses BAT activity on PET-CT, enhances meal induced energy production but reduces thermogenesis.

Conclusions: GC suppresses the function of human BAT. The enhancement of energy production in the face of a reduced thermogenic response suggests that GC reduces the dissipation of energy as heat, enhancing deposition as energy stores after nutrient intake. This may contribute to the development of obesity by GC.

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Pharmacological strategies for the prevention of preterm birth

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It has been known for decades that intrauterine infection accompanied by intraamniotic inflammation is a major cause of early preterm birth (PTB) and its associated neonatal morbidity and mortality. However, clinical trials of antenatal antibiotic therapy to prevent PTB have, to a large extent, been unsuccessful. Recent microbiome studies have profiled the bacteria associated with intrauterine infection, finding the presence of many different bacteria in the amniotic cavity with preterm deliveries; they have also reinforced the fact that inflammation can occur without the presence of bacteria in the amniotic cavity, and that bacterial infection is much more benign in the absence of an inflammatory response.

Collectively, these studies highlight the essential therapeutic requirements for successfully preventing infection-inflammation associated PTB: 1) antibiotic therapy that effectively treats the intrauterine infection, and 2) anti-inflammatory therapy that suppresses the sterile or anti-bacterial inflammatory response. Few researchers have appreciated the clinical importance of delivering dual antimicrobial/anti-inflammatory therapy and the risks of treating one but not the other.

The antibiotics that have been evaluated to date have either lacked the ability to treat the major organisms known to cause PTB, or have lacked the ability to reach fetal and amniotic tissues and fluids and treat the infection at its source. Moreover, while several anti-inflammatory therapeutic strategies have been proposed and evaluated in animal models, concerns regarding therapeutic efficacy and safety have hindered major advances in this area.

We have conducted studies of a novel 4th generation macrolide antibiotic, solithromycin, which we believe it is the first antibiotic capable of effectively treating all major PTB-associated bacteria within the amniotic cavity while also suppressing inflammation. We have also evaluated the benefits of intraamniotic delivery of cytokine signalling inhibitors in conjunction with antibiotics, using a pregnant sheep model. The combination of these two therapies may at last provide a successful pharmacological strategy for preventing a significant proportion of preterm deliveries.

Novel targets for infection- and inflammation-induced preterm birth

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Novel Mechanisms for Seminal Fluid Signalling in Reproduction

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The male has generally been viewed as having one key function in the reproductive process – to provide a single sperm to fertilize the oocyte. However, studies indicate the male contribution is more complex with effects of seminal fluid influencing pregnancy success and contributing to subsequent fetal development and offspring health. Critical to the female response to seminal fluid is the establishment of a tolerogenic immune environment, dependent on regulatory T cells (Treg cells) raised against paternal antigens, whose functions are to suppress inflammation and immune rejection responses. Factors in the seminal plasma fraction including TGFβ family members have been identified as key signalling agents, but these don’t fully account for the female response. Our research is focused on identifying novel signalling molecules in seminal fluid and determining their impact on the reproductive process. Key signalling factors we have identified include the TLR4 signalling pathway, which is activated by molecules in seminal fluid at coitus and is required for the induction of the key peri-conception cytokines Csf3, Cxcl2, Il6 and Tnf at coitus. Additionally, our studies have demonstrated that components of sperm are required for the complete female response to seminal fluid at coitus and are also involved in the induction of immune-regulatory miRNAs, including miR223. Of these miRNAs, we have demonstrated that miR223 contributes to the expansion of Treg cells following coitus and a deficiency in this miRNA alters pregnancy outcomes. As events around the time of conception have a profound impact over the course of pregnancy, a comprehensive understanding of seminal fluid function can increase our understanding of how infertility, miscarriage and disorders of pregnancy arise, and how the health of the child after birth has origins arising from both maternal and paternal determinants.

Chlamydia infections in male koalas; impacts on spermatogenesis and reproductive outcomes

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Chlamydial infections are a major cause of morbidity and mortality in koalas in South East Queensland and Northern New South Wales. Infections are usually associated with conjunctivitis (blindness) and infertility in female animals and just like human infections are considered to be predominantly a female problem. However many male koalas are PCR positive for chlamydia, mainly C. pecorum and chlamydia can be isolated from many parts of the male reproductive tract in these animals including the testis, the epididymis and the prostate. Semen collected from these animals is also PCR positive for chlamydia in many cases. Similar to findings in humans and mice, sperm from infected male koalas show signs of DNA fragmentation, which is likely to compromise fertility following either natural mating or the use of banked sperm for artificial insemination. Based on these findings we are exploring methods to clean semen of chlamydia prior to preservation in order to maintain high quality sperm banks as a resource to maintain and/or rescue species genetic diversity. Our group has also developed vaccines for both female and male koalas and the effect of these vaccines on chlamydial infection of the male reproductive tract and subsequent sperm quality is being investigated as an alternative to antibiotic treatment. A multidisciplinary approach to studying the effects of chlamydial infection on fertility in male koalas is required to protect the future of this iconic native marsupial and studies in the koala have the potential to provide important new insights into how chlamydial infections may compromise human male infertility and potentially male infertility in livestock species such as pigs and cattle.

New regulators of mammalian sex determination and gonad development

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Disorders of sexual development (DSDs) are surprisingly common. They range from mild genital abnormalities to complete sex reversal. The cause of these problems is often a failure of the complex networks of gene regulation that drives the differentiation of testes and ovaries respectively. While recent data has significantly advanced our understanding of the molecular and cellular processes of testis and ovary differentiation, many DSDs still remain unexplained at the molecular level, suggesting that important factors remain to be discovered. Past studies have focussed on the discovery and characterization of genes that are sexually dimorphically expressed, based on the assumption that a factor involved in testis differentiation is not expressed in the ovary and vice versa. We have now identified genes that are expressed in both the developing testis and ovary, for which null mutation in mice surprisingly lead to a very specific gonadal phenotype. These knockout mice display...
male-to-female sex reversal as well as a failure or a delay in ovarian germ cell differentiation. These data highlight the fact that by limiting our investigations to sexually dimorphically expressed genes we might miss important regulators.

Temperature-dependent sex determination in a variable world: mechanisms of a plastic response?
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For animals with sex determination linked to developmental temperature, variation in climate impacts population persistence and creates dynamic evolutionary pressures. Sex ratios may vary across space and time, depending on the climate, yet many species also appear to exhibit behavioural or physiological plasticity that minimizes sex ratio biases. This plasticity may be selected for in response to viability selection or frequency-dependent selection on sex, and would aid in the short-term persistence of populations under climatic change. Therefore, understanding the mechanistic basis of genetic and environmental influences on sexual differentiation informs the evolutionary history of a species and its risk of extinction.

Defining the mechanisms responsible for establishing and maintaining a pool of high quality oocytes.
Karla Hutt

Female fertility and reproductive longevity are heavily influenced by the number and quality of available oocytes, which are stored in the ovary as primordial follicles. While our understanding of the regulation of oocyte number and quality is far from complete, growing evidence indicates a key role for the intrinsic apoptosis pathway, which is controlled by the relative levels and activities of the members of the B-cell lymphoma-2 (BCL-2) family. Both pro and anti-apoptotic members of the BCL-2 family have been shown to play important roles in controlling the number of primordial follicles initially established in the ovary at birth, as well as the number of primordial follicles maintained throughout reproductive life. This talk will cover the relationship between the intrinsic apoptosis pathway, primordial follicle number, female fertility and duration of the female fertile lifespan. The role of apoptosis as a quality control mechanism will also be discussed. In particular, recent work suggests that there are two key periods of quality control within the ovary involving the apoptotic elimination of oocytes: the first occurs during embryonic life prior to the establishment of the initial ovarian reserve of primordial follicles, and the second coincides with the onset of puberty and early adulthood. It is hypothesized that apoptosis is required to ensure that only the highest quality oocytes are available for ovulation and perpetuation of the next generation.

Monotremes provide novel insights into mammalian reproduction and disease
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Comparative genomics is important for our understanding of the evolution of the molecular basis of mammalian biology but can also identify disease causing changes in genes and whole pathways. Our work on the iconic Australian egg-laying mammals has provided novel insights into the fascinating biology of the platypus and echidna and revolutionised our understanding of the origin of our own sex chromosomes. This has also led to the identification and characterization of novel genes involved in monotreme reproductive biology which are also candidate genes for reproduction in other mammals. Comparative analysis can also further our understanding of human disease as evidenced by our work on the piRNA pathway in the mammalian ovary and ovarian cancer. The piRNA pathway is important for various aspects of germ cell biology and genome integrity. Based on our previous work that showed expression of piRNA pathway genes in mammalian ovary we investigated the expression of these genes in ovarian cancer and found overexpression associated with increased malignancy. We investigated if this change in piRNA pathway activity is reflected in signature changes in small RNA profiles. Small RNA sequencing of the early and late tumours revealed less than 1% of known piRNAs present. This is consistent with other cancer small RNA datasets where the proportion of known piRNAs was also low but extensive mapping to IRNA fragments and other RNAs was observed. This raises the possibility of other, yet unknown roles of piRNA pathway genes potentially in the regulation of translation. In order to better understand the direct roles of this pathway in ovarian cancer progression we currently overexpress those pathway genes in ovarian cancer cell lines and measure effects on invasiveness, proliferation, stemness and epithelial to mesenchymal transition.
Growth hormone doping in sport
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Perceived anabolic benefits of certain hormones, such as growth hormone, have fuelled their abuse among both competitive and recreational athletes. Although growth hormone increases muscle mass, majority of that is an increase in extracellular fluid and not the functional muscle mass. In recreational athletes, growth hormone does not have major effect on muscle strength, power or aerobic capacity, but stimulates anaerobic exercise capacity. As growth hormone and testosterone interact to promote anabolic effects, many athletes abuse both of these hormones. In scientific literature, major emphasis is placed on doping detection, whereas detrimental effects of doping agents on athlete’s health are seldom discussed. Most of the doping agents exert serious side effects, especially when used in combination, at high doses, and for long duration. The extent of long-term health consequences by hormone doping is likely to be substantial.

Going downstream – how does GH binding activate JAK2
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The growth hormone receptor (GHR) has been the archetype for the class I cytokine receptor family. The original paradigm for how growth hormone (GH) binding to the GHR led to activation of the associated JAK2 was based on biophysical and structural studies over 20 years ago which showed that growth hormone binding to the extracellular domain of the receptor occurred in a sequential fashion, first binding to a high affinity site on a single receptor (site 1) and then binding to a lower affinity site (site 2) on a second receptor. We have recently defined a new paradigm by demonstrating a complete mechanistic model for JAK2 activation by the pre-dimerized GHR. We utilised multiple approaches to define this model including FRET to monitor positioning of the JAK2 binding motif, leucine-zipper receptor constructs to control receptor transmembrane (TM) helix position, atomistic modelling of TM helix interactions and docking of JAK2 kinase and inhibitory pseudokinase crystal structures with an opposing pair in trans (1). Surprisingly, we identified that receptor activation induces a separation of its JAK2 binding motifs which leads to removal of the pseudokinase domain from the kinase domain of the partner JAK2 and pairing of the two kinase domains, facilitating trans-activation. This model may represent a common mechanism to other class I cytokine receptors.

Regulation of pulsatile growth hormone secretion: Lessons from the mouse
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The production and release of GH is regulated by numerous feedback mechanisms, converging at the level of the hypothalamus and anterior pituitary gland. Within this extensive network, stimulatory Growth Hormone Releasing Hormone (GHRH)- and inhibitory somatostatin-expressing neurons are thought to predominantly modulate the characteristic patterned release of GH. This pattern of GH release (referred to as pulsatile GH release) is conserved across all species characterised to date. Modulation of GH release via these mechanisms ensures that the release of GH closely matches physiological requirements that are central to optimal growth, metabolic and reproductive needs.

While mechanisms that control GH patterning are seemingly well-defined, our understanding of the mechanisms that converge between the control of GH release, appetite and reproduction are poorly understood. Using the mouse as a model, we are currently assessing the integrative role of neuronal and peripheral regulators of food-intake and reproductive function in modulating the patterned release of GH. I will highlight key interactions between orexigenic Neuropeptide-Y (NPY) expressing neurons and hypothalamic neurons central in the control of GH release. While emphasizing the fundamental role of NPY-expressing neurons in regulating GH release relative to meal-provision, recent observations demonstrate key physiological adaptations that may override this interaction. Indeed, compelling unpublished evidence from the lactating mouse suggests specific maternal adaptations whereby enhanced GH release coincides with increased orexigenic actions of NPY neurons. While still under investigation, it is thought that this maternal adaptation occurs in response to an elevation in prolactin release that is central to lactation. This maternal adaptation is thought to ensure enhanced GH release to meet maternal metabolic requirements to the developing offspring. Discoveries highlight novel interactions within the GH axis, incorporating key mechanisms that regulate appetite and reproduction.
GH in pregnancy – how does it change and what is it doing?

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The growth hormone (GH)-insulin-like growth factor axis plays a critical role in fetal and placental development and the maternal adaptation to pregnancy. Despite this, its regulation in pregnancy is not well understood. In humans, placental secretion of GH suppresses pituitary GH secretion and replaces the pulsatile pattern of early pregnancy with stable moderate-high concentrations in the second half of pregnancy. Placentae of most mammals do not express GH, but do express hormones that regulate GH, including ghrelin and GHRH. How GH changes during pregnancy has been characterised in only a few species – in rat and pig circulating GH remains pulsatile throughout pregnancy, and we have recently demonstrated changes in patterns of pulsatile GH profiles during murine pregnancy. Fetal growth and placental function can be promoted by maternal administration of GH in non-human species, whilst responses appear to depend on the pattern of administration as well as nutritional status. This suggests that in pregnancy as in non-pregnant animals, GH action depends on patterns of exposure as well as concentrations. Maternal GH probably acts at least in part via stimulation of IGF1, which also promotes placental development and subsequent function. Manipulating the maternal GH-IGF1 pathway, particularly in early pregnancy during placental development, may therefore be an effective approach to improve fetal growth and pregnancy outcomes.

Assessment and management of obesity in Polycystic Ovary Syndrome

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Publish consent withheld

TGFB1 is a key regulator of mammary gland macrophages during development and tumorigenesis

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Macrophages are highly versatile cells capable of diverse roles in development, homeostasis and immunity. Transforming growth factor beta 1 (TGFB1) is a multi-functional cytokine that regulates cell proliferation, apoptosis and immune system responses. In the breast, TGFB1 is mainly produced by mammary epithelium, where it co-localises with macrophages and regulates macrophage functions. This study sought to investigate the role of TGFB-regulated macrophages in mammary gland development and tumorigenesis utilising a double transgenic (Clns-rTTA x TetO-TgfbrII) mouse model, whereby a dominant negative TGFB receptor is activated specifically in macrophages and attenuates TGFB signalling. Impaired TGFB signalling in macrophages resulted a 50% increase in macrophages invaded into the mammary epithelium, and the abundance of iNOS+ (“M1”) and CCR7+ (“M1”) stromal macrophages was increased by 110% and 37% respectively (p<0.05). Susceptibility to development of mammary gland cancer was investigated by challenging the transgenic mice with DMBA carcinogen. Double transgenic mice with impaired TGFB signalling in macrophages were more resistant to mammary tumour induction compared to control mice, with tumour incidence decreased and tumour latency increased (p<0.05). Immunohistochemical analysis of human non-neoplastic breast tissue revealed that macrophages may be similarly regulated by epithelial cell-derived TGFB1 in humans as they are in mice. There was an inverse relationship between abundance of TGFB1 protein and CD68+ macrophages invaded into mammary epithelium (R^2=0.26; p=0.027), and a positive relationship between abundance of TGFB1 and stromal CD206+ (“M2”) macrophages (R^2=0.33; p=0.013). These findings suggest that the mammary epithelium of mice and humans directs the phenotype and function of adjacent macrophage populations, and that TGFB1 is a key cytokine in this crosstalk. Specifically, epithelial cell-derived TGFB1 appears to reduce the abundance of “M1” macrophages involved in immune surveillance, and increase the abundance of immune tolerant “M2” macrophages, leading to increased susceptibility to breast cancer.
Twists and Turns: Balanced Wnt signalling is essential for epididymal coiling.

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Organ shape and size is an important determinant of their physiological functions. How tubal organs get their shape is a fundamental question in biology. Mouse epididymis (Wolffian duct) goes through remarkable changes during development, where almost a meter long duct fits into a small space by the extensive coiling of epithelial cells. The Wolffian duct develops as a simple straight tube, which after 15.5 day post coitum, undergoes coiling to form a highly convoluted tube. Genetic ablation studies in mouse models have shown that androgen receptor signalling is not essential for epididymal coiling, suggesting that other autocrine and/or paracrine signals play an important role in determining epididymal shape.

Canonical Wnt signalling is involved in the development of various organs and its deregulation leads to developmental defects and cancer. To understand the physiological significance of the Wnt pathway in male reproductive tract organogenesis, we studied real time changes in Wnt activity during different stages of development using a Wnt reporter mouse model. We detected active Wnt signals across the epithelium of the Wolffian duct. However, in adult mice, Wnt signalling was limited to a few regions of epididyma. To determine the functional importance of this pathway, we performed pharmacological suppression of Wnt signalling in organ culture system and showed that inhibition of this pathway results in uncoiled epididymis. Next, we developed a novel epididymal specific doxycycline regulated Cre mouse model. In this model, Cre expression is controlled by doxycycline and is limited to the epididymal/Wolffian duct epithelium, but is absent in testis. Using this Cre reporter system, we developed two triple transgenic mouse models with aberrant Wnt signalling in epididymis/Wolffian duct. Examination of epididymis from these mouse models revealed uncoiled ductal epithelium. Collectively, our results have established that a precise regulation of Wnt signalling is essential for epididymal coiling and development.

Gone with the Wnt: Unopposed oestrogen leads to endometrial cancer by regulating Wnt signalling.

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Endometrial cancer is one of the most prevalent and invasive gynaecological malignancies of the female reproductive tract, affecting 1 in 75 Australian women by the age of 75. Based on histology and clinical features it can be divided into type I and type II cancers. Type I endometrial cancer is caused by unopposed oestrogen action and constitutes about 90% of all endometrial cancers. 30% of these endometrial cancer cases show nuclear accumulation of β-catenin, which is indicative of the overactivation of Wnt signalling. How oestrogen drives the endometrial carcinoma along with hyperactivation of Wnt signalling is currently unclear.

To study this, we have developed a mouse model with doxycycline regulated cre expression limited to the uterine epithelium. Using this approach we developed a mouse model with hyperactive β-catenin signalling. Analysis of these mice revealed that overexpression of Wnt/β-catenin signalling in uterine epithelium leads to endometrial hyperplasia and carcinoma in situ, known precursors of endometrial cancer. To understand how steroid hormones affect endometrial carcinogenesis, we ovariectomised mutant mice and allowed them to rest for 14 days to remove traces of circulating hormones. After this rest period, hormonal pellets (E2, E2+E4 and vehicle) were subcutaneously inserted in mice (N=4/group). Examination of uteri from these mice after one month of treatment revealed development of endometrial cancer in E2 treated group. Co-treatment with progesterone suppressed endometrial cancer development. However, cystic growth and hyperplasia were still observed. Uteri from vehicle treated group showed endometrial hyperplasia but no cancer. In summary, we have shown that synergistic action of unopposed oestrogen along with dysregulation of Wnt signalling in uterine epithelium leads to endometrial carcinoma. This explains why human patients with type 1 endometrial cancer harbour activating mutations in the Wnt pathway.

Impaired embryo development due to oxidative stress can be normalised by BGP-15 treatment in vitro

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Oxidative stress (OS) is caused by excessive production of reactive oxygen species (ROS) and/or impaired antioxidant defence mechanisms. In oocytes, exposure to high ROS is associated with defective embryo development, apoptosis and embryonic arrest. The aims of current study were to determine 1) the mechanisms by which OS in cumulus-oocyte complexes (COCs) impairs subsequent embryo development and 2) the ability of the drug BGP-15, a ROS inhibitor, to alleviate any cellular defects. To induce OS, mouse ovulated COCs were treated acutely with hydrogen peroxide (H2O2; 50mM for 30 minutes), followed by in-vitro fertilisation (IVF) and embryo development assessments. These included differential staining to examine allocation of cell numbers, CM-H2DCFDA to measure intracellular ROS levels, JC-1 to assess mitochondrial membrane potential (MMP) and quantification of mitochondrial DNA (mtDNA) copy number to assess replication. Exposure of COCs to
H$_2$O$_2$ significantly impaired cleavage rate following IVF (p<0.05) but not subsequent blastocyst formation. BGP-15 treatment improved blastocyst development (p<0.05) particularly in embryos from H$_2$O$_2$-treated COCs. Differentiation was altered as shown by a reduction in cell numbers in the embryos generated from the H$_2$O$_2$-treated COCs; and this was normalised with BGP-15 treatment. ROS levels in embryos from H$_2$O$_2$-exposed oocytes were not different compared to control embryos, but were reduced by BGP-15 treatment (p<0.05). MMP and mtDNA copy number in the blastocysts generated from the H$_2$O$_2$-treated COCs were decreased compared to the control (p<0.05). BGP-15 did not alter MMP, but was able to restore mtDNA copy number in these embryos. These results demonstrate that COCs are acutely responsive to OS which impairs cleavage rates as well as embryo differentiation potentially via effects on mitochondria. Importantly, BGP-15, a drug currently in human clinical trials, is able to alleviate the effects of OS in oocytes and improve embryo development.

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**Progesterone regulates regulatory T cell abundance and phenotype in the hormone environment of early pregnancy**

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The fetus is antigenically distinct from the mother and therefore the maternal immune system must establish immunological tolerance towards the fetus to support pregnancy. Maternal tolerance is primarily mediated by a specialised subset of CD4$^+$ T cells known as regulatory T (Treg) cells. An absence or reduced function of Treg cells during the peri-implantation period leads to pregnancy loss in mice and is implicated as a cause of infertility and pregnancy disorders in women. The pregnancy hormone, progesterone (P4), has potent immunosuppressant effects which act through the nuclear progesterone receptor (PR), which is expressed by Treg cells. We hypothesized that P4, through PR, regulates Treg cell abundance and phenotype in the hormone environment of early pregnancy. A hormone replacement model was used, whereby mice were ovariectomised (OVX) and treated with estrogen (E2)+P4. Using flow cytometry, an increase in the number of CD4$^+$ T cells and CD4$^+$CD25$^+$Foxp3$^+$ Treg cells in the uterus-draining lymph nodes (LN) and spleen was observed in mice treated with E2+P4 compared to control treatment groups. Additionally, female PR null mutant mice (PR$^{-/-}$) and wild type mice (PR$^{+/+}$) were OVX and treated with E2+P4. When compared with PR$^{+/+}$ mice, PR$^{-/-}$ mice had decreased frequencies of Treg cell in the uterus-draining LN. To investigate the role of P4 in regulating Treg cell phenotype, splenocytes were isolated from mice and cultured under conditions to polarize differentiation towards T$_{h1}$ or T$_{h17}$ cells in the presence or absence of P4. When CD4$^+$ T cell cytokine expression was subsequently measured using flow cytometry, P4 was found to repress IFNγ expression in Treg and T effector cells. Collectively, this work demonstrates that P4, potentially acting through PR, can regulate Treg cell abundance and cytokine production, which may be important in the establishment and maintenance of competent maternal tolerance during pregnancy.

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**The significance of aspartate in regulating blastocyst glucose metabolism**

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Aspartate is the most highly consumed amino acid by the mouse blastocyst and is involved in the regulation of carbohydrate metabolism by acting as the rate-limiting factor of the malate-aspartate shuttle. Furthermore, data on global amino acid profiling reveal that aspartate consumption by blastocysts is significantly higher in groups of kinetically faster embryos. The aim of this study was to determine how aspartate consumption affects blastocyst metabolism. As metabolism of these substrates has been shown to be different in kinetically different embryos, the mRNA levels of genes involved in aspartate and carbohydrate metabolism were also determined.

We developed an ultramicrofluorescence assay to measure aspartate uptake by individual mouse blastocysts over a range of aspartate concentrations. In vivo day 4 blastocysts were flushed from CBAxC57BL/6 mice and incubated in drops of medium over increasing aspartate concentrations (0 mM, 0.1mM, 1.0mM, 10.0mM). Aspartate consumption increased with respect to concentration (P<0.001). Glucose uptake, standardized to blastocyst cell number increased in proportion to aspartate concentration (P<0.01), while lactate production displayed a similar trend (P<0.08). These alterations in glucose and lactate levels did not affect the overall glycolytic rate (percentage of glucose forming lactate).

In vitro fertilized CBAxC57BL/6 zygotes were cultured individually in a time-lapse incubator. Blastocysts were divided into quartiles based on 2-cell cleavage time and gene expression assessed by real-time PCR. Results showed that Slc2a1, PKM and GOT1 mRNAs were expressed at significantly higher levels in kinetically slower blastocysts. This suggests a compensatory response in metabolic regulation, given our previous data showing that slower, less viable, blastocysts consume less glucose and aspartate (Lee et al., 2015 Hum Reprod 30: 543-552).

These findings demonstrate that aspartate is involved in the regulation of glucose metabolism and embryo kinetics. Aspartate uptake may therefore be used as a potential biomarker for embryo selection to increase IVF success outcomes.
A common progenitor pool in the foetal ovary specifies granulosa cells of medullary and cortical ovarian follicles

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Differentiation of the foetal gonad into an ovary or testis initiates sexual development of the individual and provides the foundation for ongoing reproductive health. Formation of a testis or ovary from the bipotential gonad is initiated by the specification of Sertoli and granulosa cells, respectively. These supporting cells interact with germ cells to direct germine development and ensure regulated gametogenesis and fertility in the adult. For many years, granulosa and Sertoli cells have been considered to derive from a common precursor cell in the foetal gonad. However, recent data suggest that granulosa cells may be derived from distinct precursor populations, with one providing the medullary follicles in the early ovary, and the other providing the cortical follicles, which support lifelong fertility. This study demonstrates that both medullary and cortical granulosa cells are derived from a common precursor. The initiation of ovary development is characterised by entry of a population of granulosa cells into mitotic arrest and contribution of these cells to follicular structures. In addition, a population of pre-granulosa cells are maintained in a proliferative state and contribute to cortical follicles. We provide evidence that although derived from common precursor cells, these pre-granulosa cell populations are regulated through differing activities of FOXL2 and β-catenin. This study provides novel insights into the cellular and molecular origin of granulosa cells and suggests that β-catenin regulates a proliferative pre-granulosa progenitor cell pool, while FOXL2 regulates terminal differentiation of granulosa cells as they assemble into follicles.

Adverse effects of the hyperandrogenic environment on follicle dynamics in culture: a model of Polycystic Ovary Syndrome (PCOS)

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Polycystic Ovary Syndrome (PCOS) is a common cause of infertility, affecting 6-10% of women worldwide. Despite advances such as IVF & IVM, PCOS women seeking fertility via ART achieve fewer pregnancies per egg retrieved, reflecting suboptimal oocyte functional viability. Hyperandrogenism is a key feature of PCOS, but its role in PCOS subfertility remains unclear. Using our optimal mouse model [1], and by combining it with androgen insensitive female (homozygous AR knockout) rats [2], we provide strong experimental model evidence that AR signalling may be important in the origins of PCOS. The present study investigated the mechanisms of defective follicle selection and ovulation in PCOS using an in vitro follicle culture model. Early preantral (EP, 100-150µm), late preantral (LP, 151-200µm), small antral (SA, 201-250 µm), large antral (LA, 251-350µm) and preovulatory (PO, 351-450µm) follicles were isolated from control or PCOS mice and cultured individually. Over 5 days in culture, preantral and antral follicles from PCOS ovaries displayed decreased growth rates, whilst PO follicles exhibited a significant increase in growth rate compared to non-PCOS controls (p<0.01). Preantral follicles from PCOS ovaries maintained health and morphology with normal oocyte:follicle size ratio. By contrast, SA, LA and PO PCOS follicles all exhibited decreased oocyte:follicle ratio (p<0.05), reduced health and survival rates (p<0.01). These findings show that although the earliest stage (preantral) follicles from PCOS mice exhibit slower growth, they retain normal health and survival unlike all later stage follicles. Despite removal from the in vivo hyperandrogenic environment, later stage PCOS follicles display significantly reduced growth rates, indicating that prolonged exposure to androgen excess induces sustained, intrinsic follicular defects. These findings implicate a number of testable hypotheses for the future improvement of ART for PCOS patients, with particular focus on the early culture of preantral follicles.

BMP15 and GDF9 increase intracellular cyclic AMP concentration and junctional protein mRNA expression in granulosa cells

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Adherens junctions, tight junctions and gap junctions are at the nexus of intercellular communication within the ovarian follicle. Adherens and tight junctions establish and maintain the follicle’s 3D architecture while gap junctions mediate somatic cell-somatic cell and somatic cell-oocyte bidirectional communication. The oocyte-secreted factors (OSFs) growth differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP15) have been implicated in the regulation of somatic cell-oocyte gap-junctional communication. The regulation of adherens and tight junctions and their role in oocyte and follicle development are poorly understood. This study aimed to assess the role of BMP15 and GDF9 in regulating intracellular cyclic AMP (cAMP), and to determine if OSFs control mRNA expression of gap junction, adherens junction and tight junction genes. We used the human granulosa cell line COV434, mass-spectrometry and RT-qPCR to obtain our readouts. Firstly, cells were treated with hBMP15 (100ng/ml), mGDF9 (100ng/ml) and BMP15 + GDF9 (50ng/ml each) for 1h. Compared with controls, BMP15 and BMP15 + GDF9 tended to increase intracellular cAMP (1.4 ± 0.1, 1.7 ± 0.09 and 2.2 ± 0.3 pmol/mg protein, respectively; P<0.06), while there was no effect of GDF9 alone. A 24h time course experiment with GDF9 (25ng/ml) or dbcAMP (250µM) did not demonstrate an effect on mRNA expression. Conversely BMP15 (25ng/ml) increased mRNA expression of genes encoding vimentin, connexin43, zona occludens-1 and claudin at 6, 9 and 24h of culture. Vimentin encoding mRNA expression increased in a dose-responsive manner to increasing concentrations of BMP15 (range tested 0-100ng/ml; P<0.01). Vimentin, connexin43 and zona occludens-1 encoding mRNA increased with 6.25 and 100ng/ml of GDF9 at 24h (P<0.05). These results suggest that OSFs regulate granulosa cell mRNA expression of cytoskeletal vimentin and the mRNA of proteins specific to gap junctions (connexin43) and tight junctions (zona occludens-1 and claudin). Cyclic AMP may regulate intercellular communication via post-transcriptional mechanisms.

mTORC1 hyperactivation induces the ovarian phenotype of PCOS

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Polycystic ovary syndrome (PCOS) is the most common endocrinopathy in Australia, affecting 12-21% of the female population. Typical clinical features of PCOS include hyperandrogenism, polycystic ovaries and oligo- or anovulation. Reduced fertility is a cardinal feature of PCOS, which arises due to the hyperandrogenic endocrine environment accompanying PCOS. Several lines of evidence have shown that androgens signal through the mammalian target of rapamycin complex 1 (mTORC1) signalling pathway. However, the functional role of the mTORC1 signalling pathway within the PCOS ovary is unknown. To determine if hyperandrogenism stimulates ovarian mTORC1 signalling, we performed immunohistochemical (IHC) localisation of known markers of mTORC1 activity in ovaries of DHT treated mice, a well-established preclinical model of PCOS. IHC analysis demonstrated increased mTORC1 activity in DHT treated ovaries compared to controls. To ascertain whether mTORC1 hyperactivation is sufficient for development of the PCOS phenotype, we devised a mouse model with ovarian specific deletion of Liver Kinase B1, a major negative regulator of mTORC1 signalling. The conditional deletion of the LKB1 gene using Amhr2-cre was confirmed by examining LKB1 protein expression in control and mutant ovaries. Histological examination of LKB1 mutant ovaries revealed massive expansion of antral follicle population, which progressively develop into the polycystic ovarian phenotype. Immunostaining for AMH and inhibins confirmed that mTORC1 hyperactivation causes antral follicle expansion without any obvious changes in the preantral follicle population. Examination of mTORC1 activity revealed hyperactivation of mTOR signalling in mutant ovaries compared to controls. To understand why mutant ovaries develop the polycystic phenotype, we examined the expression of markers of cell proliferation and cell death respectively. This analysis demonstrated the concurrent increase in cell proliferation and apoptosis within the granulosa cells of mutant antral follicles. Overall these results suggest that hyperandrogenism upregulates ovarian mTORC1 activity and thereby induces the ovarian phenotype of PCOS.

Variation in Seminal Plasma Cytokine Content in Repeat Samples from Proven Fertile Men

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Alleviating the effects of acrylamide on the male germ line.

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Humans are chronically exposed to acrylamide, a toxicant present in foods cooked at temperatures above 120°C. We have chronically administered acrylamide via the drinking water to male mice at doses of 0-2mg/kg bw/day, including doses equivalent to human exposure, for periods up to 12 months (1). Using a modified version of the comet assay we have established that such exposure leads to a time and dose dependent increase in glycidamide adducts in the DNA of spermatocytes of exposed mice (1). Acrylamide is converted to glycidamide solely via the enzyme Cyp2e1 (2). Using isolated spermatocytes, treated in vitro, we have prevented both acrylamide and glycidamide induced DNA damage with an inhibitor of Cyp2e1 (resveratrol, 0.1µM) (3).

In this current study we have examined DNA damage in the sperm of mice exposed to acute doses of acrylamide (25mg/kg/day for 5 days p.i.) during spermatogenesis and post-testicular transit. DNA damage was highest when sperm were exposed during epididymal transit (acrylamide treated 156% of control) or at the pachytene spermatocyte stage (acrylamide treated 185% of control) of spermatogenesis. This correlates with Cyp2e1 expression, as determined by immunohistochemistry. DNA damage could be reduced in vivo with resveratrol cotreatment in a dose dependent manner (10–40mg/kg/day p.).

Resveratrol regulates a number of cellular processes in addition to inhibiting Cyp2e1 metabolism of acrylamide (reviewed in 4). To identify a specific Cyp2e1 inhibitor we have modified a Cyp2e1 assay for use in 96 well plates and determined the IC50 for several proposed Cyp2e1 inhibitors including an IC50 of approximately 630µM for resveratrol. We now have the pipeline in place to identify novel, specific inhibitors of Cyp2e1 with a view to inhibiting the effects of acute exposure to acrylamide and ultimately treating chronic exposure.


The exposure of sperm to epididymal cysteine-rich secretory proteins (CRISPs) is required for full male fertility

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Within humans sperm are exposed to high concentrations of a single CRISP during epididymal maturation (called CRISP1). By contrast mice are exposed to two CRISPs, CRISP1 and CRISP4, thus raising the hypothesis that human CRISP1 function is equivalent to the combined actions of CRISP1 and CRISP4 in the mouse. In order to explore this hypothesis and to define the role of CRISPs in epididymal sperm maturation, we produced homozygous Crisp1 knockout mice and cross-bred them with homozygous Crisp4 knockout mice to generate homozygous Crisp1-Crisp4 double knockout mice. Double knockout mice produce normal litter sizes compared to wild type litter mates, however, their sperm show functional deficits. Sperm were collected from mature male mice cauda epididymides via the back flushing method and analyzed by using a computer-assisted sperm analyzer (CASA). CASA data showed that sperm from double knockout mice had significantly decreased total motility compared to that of wild type (wt) mice (74.75% vs. 83.50% respectively, p<0.01). Further, progressive sperm motility...
also significantly decreased ($p<0.01$) between double knockout mice (39.63%) and wt mice (55.75%). We also analyzed sperm parameters of Crisp4 single knockout mice, however, no significant difference was found between wt and Crisp4 knockout mice in terms of sperm motility and progressive sperm ratio. Our preliminary results have demonstrated that CRISPs function during epididymal sperm maturation in order to obtain optimal sperm function and thus fertility.

### KATNAL2, a microtubule-regulating enzyme required for male fertility

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Spermatogenesis is critically dependent on several complex microtubule structures including the meiotic spindle, the spermatid manchette and the axoneme of the sperm flagellum. Despite this, the microtubule regulatory machinery of testicular cells is largely unexplored and constitutes a potentially novel avenue in understanding many causes of male infertility. The eponymous microtubule-severing enzyme, Katanin, is a heterodimer consisting of a severing enzyme subunit, KATNA1 (p60), and an associated regulatory subunit, KATNB1 (p80). This complex acts to regulate both microtubule number and length and is proving to be especially important in the generation of male gametes. Recent work has revealed that a mutation in the p80 subunit causes sterility in male mice due to disturbances in the microtubule structures of the developing germ cells. Two vertebrate specific homologs of katanin p60 are also highly enriched in the testis, suggesting that individual katanin enzymes may differentially regulate different aspects of spermatogenesis. Indeed, mutation in one homolog, KATNA1, causes male sterility and, in contrast to p80 mutation, microtubule disruption is largely restricted to Sertoli cells. Here we have shown that the previously uncharacterised p60 homolog, KATNAL2, is also essential for male fertility. Both a point mutation and complete knockdown of KATNAL2 in mice resulted in impaired differentiation of spermatids into spermatozoa and a complete retention of sperm in the seminiferous epithelium. In the absence of KATNAL2, abnormal multiplication of the basal body was observed in spermatids followed by an absence of flagellum generation in later steps. Concomitantly, spermatids exhibited defects in head shaping. Overall our findings suggest KATNAL2 is essential for the latter steps of spermatogenesis supporting the premise that a series of katanin proteins are utilised to provide differential regulation of the various microtubule structures in developing germ cells.


### Culture Expansion of Undifferentiated Human Endometrial Mesenchymal Stem Cells Using a Small Molecule Inhibitor

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Publish consent withheld


### The Changing Faces of ‘Receptive’ Uterine Epithelial Cells after Ovarian Hyperstimulation

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A decrease in uterine receptivity after fresh IVF transfers compared to frozen transfers has been documented, however the underlying mechanisms are unknown. A recently developed rat ovarian hyperstimulation (OH) model provides a novel mechanism to study endometrial changes caused by IVF drugs.
Distinct morphological and biochemical changes in luminal uterine epithelial cells (UECs) allow blastocyst implantation. These include a loss of microvilli, deepening of tight junctions (TJs), loss of adherens junctions (AJs), disappearance of focal adhesions (FAs) and increased tortuosity of the basal plasma membrane. This study investigated these changes in UECs at the time of implantation after OH to determine how this contributes to the decrease in uterine receptivity.

Ultrastructural studies of UECs at the time of implantation in OH rats show microvilli protruding from the apical surface, similar to day 1 of normal pregnancy. While there is no glycocalyx, we also note a lack of apical ADAM17, a sheddase required for cleavage of large molecules (e.g., HB-EGF) in preparation for blastocyst implantation.

Laterally, the AJ is retained and the TJs do not deepen at the time of implantation in OH rats as in normal pregnancy. While there is no change in occludin at this time between normal and OH pregnancy, there is a loss of claudin-4 after OH, suggesting a change in the permeability of the paracellular pathway.

At the time of implantation during OH pregnancy, the basal plasma membrane is flattened and contains numerous FAs with fewer morphological caveolae. There is a corresponding increase in paxillin, a focal adhesion protein, and a decrease in caveolin-1, a protein of morphological caveolae.

Collectively, these morphological and biochemical differences between ‘receptive’ UECs after OH compared to normal pregnancy provides a mechanism for the decrease in uterine receptivity immediately following fresh stimulated IVF cycles.

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Toll-like receptor 4 is an essential upstream regulator of on-time parturition and perinatal viability in mice

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The timing and progression of normal term labour is linked with an inflammation-like response in the gestational tissues. However, the upstream signals events which initiate term labour are poorly understood. Toll-like receptor 4 (TLR4) is a receptor for endogenous damage-associated molecular patterns (DAMPs) released from the fetus and/or gestational tissues in late gestation. In this study we investigated the physiological role for TLR4 in normal term delivery, and underlying mechanisms involved. To investigate this, either Tlr4-/- females or wildtype controls were mated to males of the same genotype. In Tlr4-/- mice parturition was delayed by ~13 hours and postnatal mortality was increased, compared to wild-type controls. Inflammatory cytokines and uterine activation genes were quantified using RT-PCR in the gestational tissues in late gestation. In Tlr4-/- females delayed labour was accompanied by a lower expression of Il1b, Il6, Il12b and Tnf mRNA in the placenta, fetal membrane and fetal head. A transient delay in uterine activation genes including Ptgfr, Otxr and Gja1 mRNA was observed in the uterine and decidual tissues of Tlr4-/- females, when compared to wild-type mice. The leukocyte populations in gestational tissues were also quantified using flow cytometry. In late gestation Tlr4-/- females had a decrease in placental and fetal membrane macrophages as well as the placental neutrophils. Tlr4-/- females also showed a decline in myometrial neutrophils and dendritic cells while displaying an increase of Treg cells in the myometrium. Administration of a TLR4 antagonist to wild-type mice in late gestation could also delay parturition. Collectively these results suggest that activation of TLR4 in late gestation leads to coordination of pro-inflammatory cytokine upregulation, leukocyte recruitment into the fetal and maternal tissues and induction of uterine activation genes, leading to on-time parturition.

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Comparison and Quantitation of the uterine secretome by mass spectrometry during diapause and reactivation in the tammar wallaby

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Tammar wallabies can arrest embryonic development at the blastocyst stage for 11 months with no embryonic growth or cell death. However reactivation can be induced by removing the sucking pouch young (RYP). The blastocyst is surrounded by an acellular shell coat, so diapause and reactivation must be controlled by soluble factors in the uterine secretions. We investigated the proteins in the uterine environment during diapause and reactivation. Endometrium and uterine flushings from day 0 RPY (embryo in diapause), and reactivated stages at RPY days 4, 5, 6, 8 and 9 (expansion of blastocyst starts), day 11 and day 24 (2 days before birth) were analysed by liquid chromatography-mass spectrometry (LC-MS/MS), MALDI imaging and quantitation using iTRAQ labelling. With all 3 techniques there was a distinct difference in protein profiles in diapause compared to the reactivation stages. The increased secretory activity of the endometrium from d4 RPY is accompanied by increases in metabolic enzymes, heat shock proteins and growth factors. These were also in congruence with the results from MS imaging where significant changes were noted between d0 and d4. The d4 and d5 had distinct set of peptides which might initiate reactivation with an increase in metabolism and d6 and d8 were similar
Altered hypothalamic metabolic gene expression in obese Kiss1r knockout mice
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Kisspeptin controls reproduction by stimulating GnRH neurons via Kiss1r, its receptor. Kiss1r is also expressed in other brain areas and peripheral tissues predicating a non-reproductive role. We recently examined the role of kisspeptin in energy balance by characterising the metabolic profile of Kiss1r knockout (KO) mice. These mice develop an obese and diabetic phenotype compared to wild type (WT) littermates. Our aim was to investigate why these Kiss1r KOs develop this obese phenotype. We hypothesised that hypothalamic metabolic gene expression will be altered in Kiss1r KOs. Genes examined were neuropeptide Y (Npy) (orexigenic neuropeptide) and pro-opiomelanocortin (Pomc) (anorexigenic neuropeptide). We also examined leptin receptor (Lepr), ghrelin receptor (Ghsr) and melanocortin receptors 3 and 4 (Mc3r, Mc4r). Body weights and hypothalamic gene expression (both genders) was measured in four groups. These four groups consisted of gonad intact and gonadectomised (GNX) Kiss1r KO and WT mice prior to obesity onset (8 weeks) and at obesity (20 weeks). We observed a significant increase in Pomc mRNA in gonad intact 8 week female Kiss1r KO mice (p<0.05) and 20 week male and female Kiss1r KO mice (p<0.05) compared to WT. These changes appeared to be sex steroid dependent because GNX Kiss1r KO mice exhibit no significant changes in hypothalamic gene expression. In 20 week gonad intact Kiss1r KO mice, we examined plasma leptin, insulin and c-peptide concentrations. Higher leptin, insulin and c-peptide concentrations were seen in female Kiss1r KO mice (p<0.05) compared to WT. Overall, we have identified changes in hypothalamic gene expression in Kiss1r KO mice reflecting a compensatory mechanism to their obese phenotype. Higher leptin, insulin and C-peptide levels were evident in Kiss1r KO obese female mice consistent with their obesity. These changes suggest that kisspeptin signalling influences metabolism with implications to energy balance and obesity.

Expression of Kisspeptin, neurokinin B, dynorphin and GnIH prior to and after puberty in sheep
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Secretion of gonadotropin releasing hormone (GnRH) and gonadotropins rises at puberty, but the neurochemical basis for this is unclear. Kisspeptin is a stimulator of GnRH secretion that is essential for puberty. Neurokinin B (NKB) and Dynorphin (DYN) co-localise to kisspeptin expressing cells in the arcuate nucleus (ARC) and are respective positive and negative regulators of kisspeptin secretion. Gonadotropin inhibitory hormone (GnIH) negatively regulates GnRH cells and may play a role in the pubertal transition. We aimed to determine gene expression for these neuropeptides across puberty in sheep. Brains were harvested from male and female sheep (n=4/group) prior to (20 weeks) and following puberty (32 weeks) and gene expression was quantified by in situ hybridisation. Kisspeptin cell number increased (P<0.05) during puberty in females but was reduced (P<0.05) in males in caudal ARC. Kisspeptin (Kiss1) gene expression was increased (P<0.05) by castration after puberty in males. Expression of NKB was unchanged across puberty, with no effect of castration. DYN cell number was higher (P<0.05) after puberty, but was reduced (P<0.05) by castration in males. GnIH mRNA expression decreased (P<0.05) after puberty in females but increased (P<0.01) in males and was reduced (P<0.01) by castration in males.

These data provide no definitive evidence for post-pubertal upregulation of either kisspeptin gene expression in ARC or NKB, the putative stimulator of kisspeptin secretion. Changes across puberty in DYN expression do not explain the rise in GnRH/gonadotropin secretion that occurs at this time. The reduction in GnIH expression across puberty in females is consistent with activation of reproductive function at puberty, but this is not seen in males. Castration effects in early post-pubertal animal differ from those recorded in adult animals. We conclude that changes in the expression of the genes examined in this study do not explain the pubertal process in sheep.
Can kisspeptin treatment be used to advance/induce ovulation in anoestrous bitches?
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Scheduling canine breeding is particularly difficult. Kisspeptin infusion in other species has stimulated LH secretion and triggered ovulation. The purpose of this study was to gain evidence as to the feasibility of using kisspeptin treatments as a means of stimulating follicular development and hence ovulation in anoestrous bitches. Five pairs of clinically normal, mature anoestrous Greyhounds were used in the study, with each bitch in a pair randomly allocated to receive either treatment (kisspeptin injections; 0.5mMol/kg/injection) or control (normal saline; 0.5mL). Injections were given intravenously every 4h for 10 days. To investigate the effect of treatment on pulsatile LH secretion, blood samples were collected every 10 minutes for the first 8 hours with the first injection given after 2h. Samples were then collected every 12h until the completion of the study to determine if an LH surge was generated. An LH assay was performed using a commercial ELISA kit. Preliminary results indicate little increase in LH pulsatility and no LH surge following kisspeptin treatment, a result supported by post-trial serum progesterone measurements. Despite this, the ovaries from treatment dogs showed follicular development with numerous antral follicles, whilst the ovaries of the controls were smooth without any structures. Two of the treatment dogs entered pro-oestrus during the treatment period, with clear signs of vulval oedema and serosanguinous discharge, supported by vaginal cytology. This may imply that the kisspeptin treatment protocol used in this study can stimulate FSH and initiate follicular development in an anoestrous bitch, but cannot induce an LH surge and ovulation. Either a higher dose of kisspeptin treatment or addition of an ovulation induction agent to the current protocol may lead to LH surge and ovulation.

Gonadotrophin releasing hormone challenge for the validation and analysis of luteinizing hormone in non-invasive urine samples from captive female southern hairy-nosed wombats (Lasiorhinus latifrons).
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Southern hairy-nosed wombats (SHNW) do not breed well in captivity and the collection of data to accurately evaluate and characterise the female reproductive cycle has been limited. The aim of this study was to biologically validate an enzyme-immunoassay (EIA) for the detection and analysis of luteinizing hormone (LH) in non-invasive urine samples. During the 2013 breeding season, four anaesthetised female SHNW received 4 µg exogenous gonadotrophin releasing hormone (GnRH, Buserelin: Intervet). Serial blood and urine samples were collected immediately before and up to three, or 72 hours respectively, post GnRH injection. All serum and urine samples were stored frozen (-20°C) until EIA hormone analysis of LH could be conducted. EIA analysis confirmed no serum LH response to 4 µg of exogenous GnRH. The trial was attempted again during the 2014 breeding season. Four females received a single 8 µg GnRH injection and two of those females received an additional 2 µg GnRH thirty minutes following the initial dose. All females responded to the higher dose. Mean baseline serum LH was 1.2 ± 0.18 ng/mL, and peaked between 1.2 and four fold ten to 17 minutes post GnRH injection (peak range 1.9 – 4.2 ng/mL). Mean baseline urinary LH was 0.064 ± 0.0101 ng/mg creatinine. LH peaked at 2.2 to 2.7 fold between two and three hours post GnRH injection. This is the first study to successfully detect and evaluate LH concentrations in urine samples in a marsupial species. The assessment of longitudinal urine samples for LH concentration will provide valuable information regarding LH metabolism and regulation, and the timing of ovulation in relation to mating and progesterone secretion, increasing our knowledge of the reproductive biology of SHNW. Further, the difference in GnRH dose rate responses may be an important consideration when developing assisted reproductive technologies for SHNW.

Steroid Biomarkers for Adrenal Diseases: What’s on the Horizon?
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The adrenal is characterized by the production of three groups of hormones, i.e. mineralocorticoids, glucocorticoids and the so-called, adrenal androgens. These steroids exhibit independent regulation and are produced within separate adrenal compartments. The zona glomerulosa, fasciculata and reticularis represent three phenotypically different cell types that produce aldosterone, cortisol and DHEA-sulfate as a result of very different steriodogenic enzyme expression profiles. Adrenal diseases that lead to adrenal steroid excess are often due to enzyme deficiencies or neoplastic processes. Both events cause a dramatic alteration in normal adrenal zonation and the expression pattern of steroid metabolizing enzymes. Recent genomic studies of normal and pathologic adrenal tissues have shown that adrenal adenomas and cancer have enzyme expression profiles that would predict the production of novel steroid hormones. Likewise, enzymatic deficiencies would predict the production of unique hybrid steroids not seen in normal physiology. Based on these studies we have expanded our use of liquid chromatography-tandem mass spectrometry (LC-MS/MS) to test the hypothesis that neoplastic adrenal tissue and CAH results
In circulating steroids that could act as biomarkers for patient diagnosis. While the concept of adrenal disease steroid biomarkers is not a new concept, the increased sensitivity and availability of LC-MS/MS has provided physicians access to panels of steroids that will improve diagnosis of common and rare adrenal diseases. This seminar will discuss the University of Michigan Adrenal Program's quest to define serum steroid biomarkers for primary aldosteronism, congenital adrenal hyperplasia and adrenal cancer.
and optimising management of individual patients. Patients with identified mutations of specific genes require a personalized approach to management that can include different strategies for biochemical testing, tumour localization, and potentially, therapeutic interventions.

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The Beta Cell in Type 2 Diabetes: From The Clinic to The Lab
Steven Kahn

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Content not available

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HENMT1 Is Required for piRNA Stability and Both Male and Female Fertility.
Moira O'Bryan

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The Piwi-interacting RNA (piRNA) pathway is an RNA silencing pathway that represses the expression of transposable elements (TE) in spermatogonia via binding of piRNAs to their complimentary RNA targets. Mammalian piRNAs are 26-31 nt in length and are 2'-O-methylated at their 3' termini. The role of piRNAs in adult male germ cells types, however, wherein the majority of piRNA sequences are not complementary to TE sequences, remains poorly defined. To address this question, and the role of piRNAs in female fertility, we have used a unique mouse model of HENMT1 dysfunction (Henmt1Pin/Pin). HENMT1 is an RNA methyltransferase that acts to add stabilizing 3' methyl groups to piRNAs. The loss of piRNAs in adult male germ cells results in male infertility characterized by TE over-expression, the precocious expression of haploid germ cell transcripts in meiotic cells and a catastrophic deregulation of the haploid germ cell program. Our data strongly suggests a role for piRNA in promoting a heterochromatic state in the regulatory regions of many spermiogenesis genes during meiosis and their necessity to set an appropriate gene expression program. Further our data show Henmt1 dysfunction in female mice leads to a sub-fertility phenotype reminiscent of premature ovarian failure in humans. Specifically, Henmt1Pin/Pin females are fertile while young, but have depleted ovarian reserves by six months of age. Further, even when young the quality of oocytes from Henmt1Pin/Pin females is significantly compromised. Collectively these data support a role for HENMT1 and piRNAs in oocyte survival during the perinatal period and in the regulation genes critically involved in adult oocytes function.

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Developmental programming of insulin resistance and diabetes
Catherine Suter

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Parental nutrition can program the metabolism of offspring, creating stable changes in physiology that may have significant health consequences later in life. We have previously reported that offspring exposed to maternal obesity in utero exhibit a latent predisposition for metabolic disease in adulthood that is associated with widespread epigenetic changes. Our most recent work demonstrates that a latent metabolic phenotype reminiscent of premature ovarian failure in humans. Specifically, Henmt1Pin/Pin females are fertile while young, but have depleted ovarian reserves by six months-of-age. Further, even when young the quality of oocytes from Henmt1Pin/Pin females is significantly compromised. Collectively these data support a role for HENMT1 and piRNAs in oocyte survival during the perinatal period and in the regulation genes critically involved in adult oocytes function.

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Understanding increased metabolic risk associated with IVF
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In vitro fertilisation (IVF) has revolutionised human reproduction and enabled millions of couples with compromised fertility to conceive children. Developmental programming is a well-recognised concept, but the long term health implications of IVF are understudied. The data that has emerged in recent years suggests that children conceived by IVF are at increased risk of developing type 2 diabetes and cardiovascular disease, later in life. However, human studies cannot separate out risk from genetics/environment. Here, I will summarise the existing evidence in humans, and in mouse models, including our own recent data in humans and in genetically identical, young adult C57Bl6J mice that were generated utilising state of the art embryo culture. Our data suggests that human and mouse IVF offspring are at increased risk of developing insulin resistance, which is considered a hallmark of these metabolic diseases. We are now working towards understanding the cause of insulin resistance
in IVF models, and whether this is a result of embryo culture, or ovarian stimulation and finally we hope to determine whether this risk can be mitigated and/or eliminated in future generations.

### Periconceptional alcohol exposure - uterine and blastocyst contributions to offspring health

**Karen Moritz**

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Perturbations around the time of conception have long lasting effects on adult health of the offspring. ~ 80% of Australian women drink alcohol around the time of conception but cease upon pregnancy recognition. We have developed a model of periconceptional alcohol exposure (PCE) in which rats are exposed to alcohol for the 4 days prior to conception until day 4 of gestation. PCE resulted in fetal growth restriction and offspring with severe adult insulin resistance, increased fat deposition, as well as renal and cardiac dysfunction. We have examined the placenta in late gestation and found striking sex-specific alterations in placental morphology suggesting impaired placental development may contribute to disease outcomes. The placenta is composed of both maternal and blastocyst derived tissues and optimal placental formation is dependent upon orchestrated interactions between embryonic trophoblast cells and endometrial cells of a receptive uterus. Our recent studies have focussed on the effects of alcohol on the maternal uterine environment and the blastocyst. At E5, following PCE, we have demonstrated altered expression of markers of receptivity including elevated expression of uterine sensitization-associated gene-1 (Usag-1) suggesting the uteri of the dams exposed to PCE become receptive earlier than control dams and may not optimally synchronize with arrival of the blastocyst in the uterus. Studies on the blastocyst have determined that PCE does not affect cell number. However, using trophoblast stem (TS) cell cultures, we found alcohol could directly affects the ability of trophoblast cells to prolifere, differentiate and interact with the maternal endometrium as evidenced by decreased expression of several trophoblast subtype-specific markers. Finally, we have determined that PCE results in widespread changes in genes that regulate epigenetic status including the DNA methyltransferases (DNMTs) in fetal organs in later gestation. Together these studies suggest that alcohol around conception has multiple effects on early developmental processes including uterine receptivity, placental development and methylation patterns which combine, contribute to the growth restriction and long term disease outcomes.

### Metabolic and circadian perturbations in liver and adipose tissue underlie programming by glucocorticoids in rats: modulation by postnatal high fat and omega-3 fatty acids

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Excess prenatal glucocorticoid exposure predisposes offspring to an adverse metabolic phenotype, including altered adipose tissue gene expression and can lead to obesity and type 2 diabetes. Clock genes are now recognized as key regulators of metabolic function in a range of tissues, but the impact of programming insults on their expression is unknown. The aim of this study was to determine if circadian variation in adipose and liver tissue expression of clock genes is programmed by prenatal glucocorticoids. Furthermore, we investigated whether postnatal high-fat (HF) diet (± omega-3 fatty acid supplementation) further modifies programmed outcomes. Rats were either untreated (Con; n=24) or dexamethasone treated (0.5 μg/ml in drinking water; Dex, n=24) from pregnancy day 13 to term (day 23). Offspring were cross fostered to untreated mothers and males were weaned onto either a standard (Std), a HF or HF, high n-3 (HFHn3) diet. After 6 months, tissues were collected at four Zeitgeber times (ZT1=8am, ZT7=2pm, ZT13=8pm and ZT19=2am; each n=8). Plasma insulin and glucose, and circadian expression of hepatic and retroperitoneal fat clock genes and PPAR genes were measured. Prenatal Dex elevated offspring glucose (p<0.05), tended to increase insulin levels (p=0.061) and suppressed adipose tissue expression and rhythmicity of Clock, Bmal1, Per1 and Per3 (p<0.01). HF consumption suppressed expression of Per1, Per2 and Cry1 (p<0.01) in adipose tissue. Supplementation with n-3 partially corrected HF-induced changes in adipose tissue clock genes (Per1, Per2, and Cry1; p<0.05). In conclusion, prenatal Dex treatment programmed perturbations in plasma glucose and insulin profiles and adipose tissue clock gene expression. While a postnatal HF diet amplified this phenotype, supplementation with n-3 reduced the impact of both programming and the HF diet. We conclude that changes in clock gene rhythmicity and glucose sensitivity may underlie glucocorticoid programming of adipose tissue metabolism.

### Exercise: how much and what are the benefits for fitness versus fatness

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*Publish consent withheld*
Exercise prescription for bone health and fracture prevention

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Exercise is widely recommended to prevent osteoporosis and reduce the risk of falls and fractures, but not all forms are equally effective. At present the optimal type, intensity, frequency and duration of exercise that can preserve skeletal integrity and prevent fractures is not known, but it is recognized that the effects of exercise on musculoskeletal health and function are modality and intensity-dependent. Regular walking alone has little or no effect on preventing bone and muscle loss, and may even increase the risk of falls and fractures. Most of the available evidence from intervention trials in middle-aged and older adults, without osteoporosis, indicates that programs incorporating weight-bearing activities, such as walking, moderate- to high- and odd-impact activities that become progressively more difficult or varied over time in combination with high-intensity progressive resistance training (PRT) can increase (1-4%) or maintain hip and spine BMD. Traditional PRT is also very effective at improving muscle strength and mass, but has mixed effects on balance and functional performance. However, high-velocity PRT (or power training), which involves rapid concentric muscle contractions, can improve muscle function, power and BMD in older adults. For falls prevention, high-challenging balance training performed for at least 50 hours in total (e.g. twice a week for 25 weeks) appears to be most effective in community-dwelling older adults. Whether exercise can prevent fractures remains uncertain because there have been no long-term and adequately powered RCTs. However, a recent systematic review and meta-analysis reported that exercise reduces overall fractures and, to a lesser degree, vertebral fractures in the elderly by up to 50% This presentation will review the latest evidence with regard to the optimal mode and dose of exercise that can enhance bone health as well as improve muscle function and reduce falls and fracture risk in the elderly.

Exercise as an intervention after IUGR

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Individuals born after intrauterine growth restriction (IUGR) are at an increased risk of developing diabetes in their adult life. IUGR impairs β-cell function and reduces β-cell mass, thereby diminishing insulin secretion. IUGR also induces insulin resistance, with impaired insulin signaling in muscle in adult humans who were small for gestational age (SGA) and in rodent models of IUGR. There is epidemiological evidence in humans that exercise in adults can reduce the risk of metabolic disease following IUGR. However, it is not clear whether adult IUGR individuals benefit to the same extent from exercise as do normal-birth-weight individuals, as our rat studies suggest less of a benefit in those born IUGR. Importantly, however, there is some evidence from studies in rats that exercise in early life might be able to reverse or reprogram the long-term metabolic effects of IUGR. Studies are needed to address gaps in current knowledge, including determining the mechanisms involved in the reprogramming effects of early exercise in rats, whether exercise early in life or in adulthood has similar beneficial metabolic effects in larger animal models in which insulin resistance develops after IUGR. Human studies are also needed to determine whether exercise training improves insulin secretion and insulin sensitivity to the same extent in IUGR adults as in control populations. Such investigations will have implications for customising the recommended level and timing of exercise to improve metabolic health after IUGR.

The Health Hazards of Too Much Sitting – What Can We Do About It?

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In contemporary society, prolonged sitting has been engineered into our lives across many settings, including transportation, the workplace, and the home. There is new evidence that too much sitting (also known as sedentary behavior – which involves very low energy expenditure, such as television viewing and desk-bound work) is adversely associated with health outcomes, including cardio-metabolic risk biomarkers, type 2 diabetes, some cancers and premature mortality. In addition to the decreased energy expenditure induced through sitting, sedentary time may also be harmful because of the prolonged absence of muscle contractile activity in the lower limbs. Importantly, these detrimental associations remain even after accounting for time spent in leisure time physical activity – which within adult populations is infrequent and very low volume. This presentation will provide an overview of recent evidence from epidemiological and experimental studies. This new evidence is beginning to make a strong case that too much sitting should now be considered as a stand-alone element within public health recommendations – particularly for reducing the risk of type 2 diabetes and cardiovascular disease.
DHH, ETV5 AND NEDD9 - Novel Targets of Sox9 in Mammalian Sex Determination
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SOX9, a DNA binding transcriptional activator, is the main regulator of mammalian testis development and plays a major role in Sertoli cell differentiation, testis development and fertility. Target genes of SOX9 have previously been characterised such as AMH, FGF9 and PTGDS but due to the high number of idiopathic 46,XY DSD cases, we suspect that there are more. Here, we utilise transcriptome analysis of E13.5 Sox9 knock-out gonads in order to identify SOX9-responsive genes in the developing testis. The candidate genes include DHH, ETV5 and NEDD9. To investigate the mechanisms of regulation of these genes, an in vitro approach was utilized. Candidate genes were validated in NT2/D1 cells by assessing their response to SOX9 overexpression and knockdown. In silico analysis of DHH and ETV5 promoter regions was used to assess the binding potential of SOX9 and sites were validated using SOX9 ChIP-seq in NT2/D1 cells, luciferase assay and EMSA. Immunohistochemistry was used to visualise the localisation of NEDD9 and ETV5 in E13.5 mouse testis in Sertoli cells. SOX9 ChIP-seq in embryonic bovine gonads was also analysed. NT2/D1 cells transiently over-expressing SOX9 or knockdown with siRNA reveals that DHH, ETV5 and NEDD9 respond significantly in the manner to which the SOX9 expression is altered. In silico analysis of DHH and ETV5 promoter regions revealed potential binding sites which were confirmed by ChIP-seq analysis of NT2/D1 cells, luciferase assay and EMSA. A peak in the bovine SOX9 ChIP-seq across the promoter region of NEDD9 also reveals a potential binding site for future analysis. Immunolocalisation of NEDD9 in the mouse testis revealed its expression in the Sertoli cell cytoplasm and ETV5 in the nucleus at E13.5. DHH, ETV5 and NEDD9 are all likely direct targets of SOX9 in the developing testis. Further analysis of NEDD9 is planned including the assessment of knockout gonads.

Surveying the epigenome landscape of the prostate cancer microenvironment: identification of estrogen receptor α as a key differentially methylated gene
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The stroma acquires molecular and functional changes in prostate cancer. This was once assumed to be a transient reaction to aberrant signalling from nearby cancer cells. Yet, there is increasing evidence that stroma undergoes more permanent changes, because non-malignant prostate fibroblasts (NPFs) and cancer-associated fibroblasts (CAFs) maintain their differences in the absence of epithelium. For example, steroid hormone receptors in tumour stroma, including estrogen receptor α (ERα), are differentially expressed between NPFs and CAFs. Therefore, we hypothesised that stroma acquires epigenetic modifications that alter the expression of steroid hormone receptors and promote tumour progression.

Primary cultures of NPFs and CAFs were established from radical prostatectomy specimens from 15 patients. In vivo tissue recombination assays were used to verify the functional differences between cells and show that CAFs, but not NPFs, induced prostate epithelial cells to form tumours. Whole genome bisulphite sequencing was used to construct the first complete epigenome map of human tumour stroma.

Our data demonstrated that NPFs and CAFs have distinct epigenome profiles with locus-specific rather than genome-wide differences. We identified ~7000 differentially methylated regions (DMRs) between CAFs and NPFs; many were at key regulatory loci and correlated with differential gene expression profiled with RNAseq. Targeted bisulfite sequencing showed that changes in DNA methylation were highly consistent between patients and could accurately discriminate between CAFs and NPFs. Of note, ESR1 which encodes ERα, was hypomethylated in CAFs. Accordingly, CAFs exhibited increased ERα expression and enrichment of an estrogen-regulated gene signature, of which CXCL12 was the most highly over-expressed gene. CXCL12 secreted by CAFs recruited CXCR4+ mast cells, activating a pro-tumourigenic loop in the tumour microenvironment.

This study shows that epigenomic changes are an underlying mechanism for the enduring differences between NPFs and CAFs. Furthermore, key epigenetically-regulated genes in CAFs, like ESR1, promote the progression of prostate cancer.
A novel class of Hsp90 inhibitors induce apoptosis in prostate tumours while minimising mechanisms of resistance.

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The molecular chaperone Hsp90 is overexpressed in prostate cancer (PCa) and is responsible for folding, stabilisation and maturation of many oncoproteins implicated in PCa progression. Consequently, targeting Hsp90 by small molecule inhibitors is a rational strategy for treatment of advanced PCa. Unfortunately, while agents such as 17-allylamino-demethoxygeldanamycin (17AAG) and AUY922 have demonstrated promising efficacy in cell lines, animal models, and tumour tissues cultured as explants, these Hsp90 inhibitors, currently undergoing clinical trials, also induce a heat shock response (HSR) in target cells. This leads to accumulation of various heat shock proteins, notably Hsp27 and Hsp70, which have cytoprotective properties and may represent an important mechanism of clinical resistance to these agents. Our research has resulted in the development of a new class of Hsp90 inhibitors that target a different domain of Hsp90 compared to previous Hsp90 inhibitor compounds and do not induce HSR. In this study we demonstrated that two promising new Hsp90 inhibitors, SM253 and SM258, do not result in elevated expression of Hsp27 or Hsp70. This was revealed by qPCR and Western blot of PCa cell lines (22Rv1, LNCaP and PC3) after 48hrs culture with DMSO (vehicle control), 17-AAG (50nM), AUY922 (25nM), SM253 (5uM), or SM258 (5uM). Furthermore, cleaved caspase-3 staining in cell lines and tumour tissues cultured as explants clearly demonstrated these novel inhibitors are capable of significantly inducing apoptosis of PCa cells at low micromolar concentrations. The efficacy of SM253 and SM258 treatment in PCa cell lines and explant tissues earmarks this new class of inhibitors for further clinical evaluation, particularly as they offer a novel strategy to target Hsp90 without inducing protein pathways implicated in drug resistance. Ultimately, this study indicates that the design and use of alternate Hsp90 inhibitors will maintain a focus on Hsp90 as a highly promising oncogenic target for PCa treatment.

Elf5 is associated with FOXA1 expression in the absence of AR and survival outcomes in triple negative cancer patients.

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Publish consent withheld

Targeting activin to prevent muscle wasting

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Activins, integral members of the transforming growth factor-β superfamily, are negative regulators of muscle growth. Elevated levels of activins in patients diagnosed with metastatic cancers are associated with marked body wasting, termed cancer-cachexia. Significantly, cachexia is observed in the majority of patients suffering advanced cancers and accountable for 25% of cancer-related mortalities. The favoured approach to combat activin hyperactivity in models of cancer-cachexia uses soluble forms of the activin type II receptors (sActRIIA/B). By binding to diverse TGF-β proteins, sActRIIA/B can increase muscle and bone mass, correct anaemia or protect against diet-induced obesity. While exciting, these multiple actions of soluble ActRIIA/IIB limit their therapeutic potential and highlight the need for new reagents that target specific ActRIIA/IIB ligands. Here, we modified the activin prodomains, regions required for mature growth factor synthesis, to generate specific activin antagonists. Initially, the prodomains were fused to the Fc region of mouse IgG2A antibody and, subsequently, “fastener” residues (Lys45, Tyr96, His97 and Ala98) that confer latency to other TGF-β proteins were incorporated. These modifications generated a reagent that potently (IC50 5nM) and specifically inhibited activin signalling in vitro, and activin-induced muscle wasting in vivo. Importantly, unlike soluble ActRIIA/IIB, the modified prodomains did not inhibit the activities of related ActRII ligands, myostatin or GDF-11. To underscore the therapeutic utility of specifically antagonising activin signaling, we demonstrate that the modified activin prodomains promote significant increases in muscle mass. Using a mouse xenograft model, we also showed that pharmacological delivery of the prodomains could prevent tumour-associated muscle wasting. Significantly, our novel activin therapeutic has exciting potential in the treatment of cancer-cachexia.
Active alternative ‘backdoor’ pathway in CAH demonstrated by urine steroid profiles

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Introduction: The classic pathways of androgen synthesis are Δ5 (17,20 lyase activity of CYP17A1; conversion of 17-hydroxypregenolone to DHEA) and Δ4 (conversion of 17-hydroxyprogesterone (17OHP) to androstenedione). In congenital adrenal hyperplasia (CAH) due to 21 hydroxylase deficiency, accumulated 17-hydroxyprogesterone is converted to pregnanediol (pdio1) (SRDA1/2; 5α reductase type 1 or 2). Pdio1 acts as a substrate for CYP17A1 with an affinity higher than 17OHP. This converts pdio1 to androsterone with subsequent conversion to dihydrotestosterone and testosterone. This alternative pathway is an efficient route of androgen production in CAH. We aim to demonstrate evidence of the alternative pathway in urine steroid profiles (USP) of CAH patients.

Methods: Urine steroid metabolites were determined using GCMS on 24 hour urine samples. All USP results over a 10 month period (2014) were collated. USPs with CAH noted on clinical history or a pattern consistent with CAH (elevated pregnanetriol) were classified as CAH. Age-matched controls for CAH USPs were selected from normal profiles. Androsterone and etiocholanolone concentrations and the Androsterone to etiocholanolone ratio (A:E) were compared between CAH and control groups.

Results: Out of 427 USP, 47 were from CAH patients (30 females, 14 males, mean age 15y, range 0 to 44). Nine were treated (suppressed pregnanetriol). Five of the untreated patients had a profile consistent with 11-hydroxylase deficiency. Androsterone and A:E were significantly higher in the untreated CAH group compared to controls (P= 0.001 and 0.01). Androsterone was significantly higher in untreated CAH than treated CAH (P=0.006). A:E for treated CAH was not significantly different from controls.

Conclusion: The active alternative pathway of androgen synthesis in CAH can be demonstrated by USP. Treatment of CAH to achieve suppression of pregnanetriol appears to suppress the alternative pathway. This suggests that the metabolites of the alternate pathway may be used in diagnosis and monitoring therapy.

Characterization of a novel human species-restricted hydroxysteroid dehydrogenase called 11bHSD1L in the hypothalamus-pituitary-gonadal axis

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Endocrine steroid hormones including estrogens, androgens, glucocorticoids and mineralocorticoids play clinically important and specific regulatory roles in human development, growth, metabolism, reproduction and brain function. The 11-beta hydroxysteroid dehydrogenase enzymes have key roles in the pre-receptor modification of glucocorticoids, modifications that directly regulate blood pressure, fluid and electrolyte homeostasis, as well as modulating metabolic and brain function. We have recently identified a novel largely uncharacterized 11bHSD-like gene on human chromosome 19q13.3, a distinct gene from the very well characterized 11bHSD1 and 11bHSD2 genes. Strikingly, a search in other mammalian genomes has revealed the complete absence of this 11bHSD-like gene in the mouse, rat and rabbit genomes. The human 11-beta-hydroxysteroid dehydrogenase 1-like protein (HSD11B1L) gene is encoded by 9 exons and analysis of EST library transcripts indicates the use of two alternate ATG start-sites in exons 2 & 3, and alternative splicing in exon 9. We have detected expression of this enzyme in tissue samples from the brain, ovary and testis. Analysis of cell-type specific expression by immunohistochemistry localizes cytoplasmic expression to ovarian Granulosa cells, testis Leydig and sperm cells, and somatotroph cells in the anterior pituitary from non-human primates and the sheep. The endogenous substrate of this enzyme is unknown but we intriguingly we show that it is very unlikely to be cortisol or cortisone.

Activation of the mineralocorticoid receptor promotes tissue inflammation in part via the peripheral molecular clock

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Activation of the mineralocorticoid receptor (MR) promotes inflammation and fibrosis. Clinical and experimental studies have shown that MR blockade is beneficial in abrogating these effects; however its use is limited due to negative side effects. Thus the identification of cell-specific MR signalling mechanisms may allow for the development of more cardiac-specific MR antagonists. We have shown that in mice null for the MR in cardiomyocytes, regulation of Per2 is lost. Per2 is a member of the peripheral molecular clock (PMC), an anticipatory “transcriptional-translational feedback loop”. Dysregulation of this pathway is
associated with cardiovascular disease and may be one potential pathway linking MR activation to cardiac dysfunction. Therefore we hypothesise that the MR regulates the peripheral molecular clock to promote dysregulation of its downstream targets that are involved in cardiac inflammation and fibrosis.

Unineprectomised 8wk old male wild type, Clock∆19+mel+ (CLK) and cardiomyocyte MR-null mice (myoMRKO) were given 0.9% saline without (VEH) or with deoxycorticosterone (DOC) 7mg/week (n=8-11). Cardiac tissue inflammation and fibrosis by immunostaining showed DOC/salt promoted inflammation and fibrosis in wild type mice. CLK-DOC mice showed elevated baseline values for inflammation and fibrosis (WTVEH vs. CLKVEH macrophages 34%, and tissue collagen 35%), but a blunted response to DOC/salt injury (Fibrosis WT vs CLK 70% vs 40%). In contrast, myoMRKO mice are protected from DOC/salt cardiac inflammation and fibrosis. We also identified differential gene expression profiles for PMC genes and MR-dependent genes in whole heart between genotypes, indicating a specific subset of PMC genes are regulated by the MR. Of note, systolic blood pressure at 8 weeks was normal in CLK-DOC mice and associated with reduced renal inflammation.

These data suggest that although disruption of the PMC promotes some cardiac remodelling, the MR can regulate the PMC in the heart to drive DOC/salt inflammation and fibrosis and potentially hypertension.

**Obesity, Diabetes and Fracture Risk**

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The effect of obesity on fracture risk is divergent; it demonstrates site-dependency, the risk being increased for some fractures (humerus, ankle, upper arm) and decreased for others (hip, pelvis, wrist). The relationship between obesity and fracture also varies by sex, age, and ethnicity. Risk factors for fracture in obese individuals appear to be similar to those in non-obese populations, although patterns of falling and the effects of increased skeletal loading are particularly important in the obese. Research is needed to determine if, and how, visceral and intramuscular adipose tissue, and metabolic complications of obesity (insulin resistance, chronic inflammation, etc.) are causally associated with bone mineral density (BMD) and fragility fracture risk.

Diabetes is also an independent risk factor for osteoporosis and fractures, although the mechanism of bone loss differs in type 1 and type 2 diabetes. In type 1 diabetes, which often has its onset during the acquisition of peak bone mass, there is a six-fold increase in fracture risk. The main effect is due to insulin deficiency, which leads to decreased bone formation, lower rates of bone modelling and remodelling, and low bone density. By contrast, in type 2 diabetes, BMD is often increased, yet fracture risk is doubled. This is due to reduced bone quality and effects of advanced glycation end-products (AGEs) on non-enzymatic collagen cross-linking to increase bone fragility. In animal studies increased bone levels of one of these AGEs, pentosidine, are associated with reduced bone strength. Serum pentosidine levels have also been associated with an increased risk of vertebral fractures in postmenopausal women with diabetes, independent of BMD. New techniques to examine bone quality in type 2 diabetes are: high-resolution pQCT (which has shown increased cortical porosity); trabecular bone score; and micro-indentation of tibial bone. In both types of diabetes, complications such as low vision, diabetic nephropathy, and peripheral and autonomic neuropathy, all increase the risk for falls and fractures. The class of oral anti-diabetic drugs known as glitazones decrease bone formation and promote bone loss and osteoporotic fractures in postmenopausal women. GLP-2 agonists decrease bone resorption and increase BMD, while DPP-4 inhibitors use is associated with decreased fracture risk and an increase in bone formation markers. SGLT-2 inhibitor use is associated with an increase in fracture risk. Dapagliflozin use does not change BTMs or BMD, but is associated with a higher risk of upper limb fractures.

In conclusion, diabetes and, to a lesser extent, obesity, are important, but neglected, risk factors for fractures. BMD is not decreased in type 2 diabetes, so the diagnosis may only be made after a minimal trauma fractures. Despite reduced bone remodelling and bone formation being features of bone disease in diabetes, all anti-osteoporosis drugs seem to be effective.

**Osteoporosis imperfecta and other heritable disorders of bone fragility: investigation and management in adults**

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Content not available
Use of QCT and trabecular bone score in the assessment of Osteoporosis
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Osteoporosis is characterised by low bone mass and micro-architectural deterioration. Bone mass is most often quantified by DXA. QCT is also used; a major disadvantage being radiation dose. The WHO T score criteria of osteoporosis are not applicable to spine QCT and interpretation is usually based on American College of Radiology guidelines. QCT bone density output of the proximal femora is similar to DXA of the hip and WHO T score criteria may be applied. There are few large longitudinal studies on QCT in predicting fracture but DXA hip data is supportive. QCT has a potential advantage of measuring structural parameters (e.g. cortical thickness) but incorporation of these parameters into clinical practise remains difficult.

Assessment of the second characteristic of osteoporosis, deterioration of bone micro-architecture, has proved more difficult than measuring bone mass. HR-pQCT can derive micro-architectural parameters in the extremities, usually tibia or radius, but radiation dose limits its application in central sites. The high resolution of HR-pQCT allows measurements of cortical porosity and thickness, and parameters of trabecular microarchitecture, which can be used to derive estimates of bone strength using voxel-based finite element analysis. HR-pQCT has proved to be a major research tool. Cost, availability, and limited data confirming validity of extrapolation to prediction of axial skeleton microarchitecture, are factors limiting current clinical application.

Trabecular bone score (TBS) derived from DXA spine images is a new parameter providing an indirect index of trabecular microarchitecture by evaluating pixel grey-scale variations. TBS is an independent predictor of fracture and has been shown to improve fracture prediction. Limitations of TBS include decreased reliability in obese patients. Recently, FRAX® has incorporated TBS into its absolute fracture risk algorithm facilitating an expansion of its role into clinical practise.

Optimal fracture prediction in the future will integrate measurements of bone mass, microarchitecture and clinical risk factors of osteoporosis.

Renal bone disease - how to assess and manage
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Content not available

ß-cell Apoptosis: The Role of Apoptosis Repressor with CARD Domain (ARC)
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Reactive oxygen species and diabetic complications
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There is increasing evidence that reactive oxygen species play a significant risk in diabetes associated complications, including micro- and macrovascular injury.

NADPH oxidases are the only enzymes which are dedicated to produce ROS. We have identified that the NADPH oxidase Nox1 plays a pivotal role in development and progression of diabetes associated atherosclerosis. Interestingly Nox4 in the vasculature appears to play a protective role. In the kidney, Nox4 appears to de deleterious for albuminuria development and renal fibrosis. Genetic deficiency of Nox4 provided renoprotection and podocyte specific Nox4 deletion also attenuated albuminuria and renal fibrosis in diabetic nephropathy. Furthermore, novel Nox inhibitors such as GKT137831 have been developed and have shown atheroprotective and renoprotective effects in intervention and prevention studies in diabetes. These drugs are currently in clinical phase IIb evaluation.

More recently, the human isoform Nox5 has been suggested to play a key role in diabetes associated complications. We are currently exploring this isoform using unique Nox5 expressing mice in either endothelial or smooth muscle cells in the context of diabetes. It is expected that novel interventions targeting Nox derived ROS will play a role in the future treatment of diabetes associated vascular complications.
Impact of HDL on Oxidative Stress in Diabetes
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Human population studies have established that plasma high density lipoprotein (HDL) cholesterol levels are associated with a decreased risk of cardiovascular disease. HDLs have a number of cardioprotective properties, including an ability to inhibit inflammation in multiple cell types involved in atherosclerotic lesion development such as endothelial cells, smooth muscle cells and macrophages.

HDLs also have anti-diabetic and anti-oxidant properties. In addition to inhibiting the oxidation of atherogenic low density lipoproteins (LDLs), HDLs and apoA-I, the main HDL apolipoprotein, also inhibit the high glucose-induced formation of reactive oxygen species in macrophages. However, HDLs and apoA-I from people with type 2 diabetes mellitus (T2DM) inhibit reactive oxygen species formation in macrophages less effectively that HDLs from healthy individuals. The attenuated anti-oxidant properties of HDLs from people with T2DM has been attributed to the non-enzymatic glycation of HDL apolipoproteins. In addition to reducing the capacity of HDLs to inhibit oxidative stress in macrophages, non-enzymatic glycation of HDL apolipoproteins also impairs the anti-inflammatory properties and other cardioprotective functions of HDLs.

Advanced Glycated Products
Mark Cooper

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Diabetes which is a state of chronic hyperglycaemia leads to increased generation of advanced glycated products (AGEs). These AGEs are chemical moieties which can have direct effects on protein structure and function but also interact with a number of well characterised receptors including RAGE to activate a range of pathways which can induce end-organ damage. By promoting oxidative stress and by activating intracellular signalling pathways including protein kinase C and NFkB these AGEs can promote fibrosis, inflammation and angiogenesis, cellular processes which lead to the classical hallmarks of diabetic complications. Increasingly, dicarbonyl intermediates such as methylglyoxal which are AGE precursors are considered to also be critical in the development of diabetic complications. A range of drugs which target the advanced glycation pathway at the level of precursors, AGE ligands or receptors have been investigated in a range of diabetic complication although currently no specific treatment has reached the stage of advanced clinical development.

Enhanced sensitivity to angiotensin II in the mesenteric arteries of late pregnant relaxin deficient mice.
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Pregnancy is associated with reduced peripheral vascular resistance, underpinned by altered vascular reactivity of the endothelium and smooth muscle of key vascular beds. Failure of the maternal vasculature to adapt correctly can lead to serious complications such as preeclampsia. The peptide hormone relaxin plays a major role in regulating the maternal renal vasculature during pregnancy, but little is known about the actions of relaxin on vascular reactivity of the mesenteric artery. Therefore, this study tested the hypothesis that vascular reactivity will be compromised in the mesenteric artery of pregnant relaxin deficient (Rln−/−) mice. The vascular responses of small first order mesenteric arteries were measured in non-pregnant (oestrus) and late pregnant (day 17.5) wildtype (Rln+/−; n=5-7) and Rln−/− (n=5-7) mice using wire myography. In Rln+/− mice, there was a significant reduction in sensitivity to the vasoconstrictor angiotensin II (All) but not the thromboxane mimetic U46619 in late pregnant compared to non-pregnant mice. In Rln−/− mice, this normal pregnancy adaptation to All did not occur, resulting in significantly enhanced vasoconstriction responses to All, which were endothelium-independent. This was not a result of altered expression of All receptors in the Rln−/− mice or an increase in reactive oxygen species as blocking with catalase, tempol and superoxide dimutase had no effect on the contractile responses to All in either genotype. Blocking nitric oxide synthase with the inhibitor L-NAME further enhanced the vascular response to All in both genotypes, whereas inhibition of prostanoid production with indomethacin significantly increased All-induced contraction in day 17.5 pregnant Rln−/− but not Rln+/− mice. In conclusion, sensitivity to All is enhanced in the mesenteric artery of late pregnant Rln−/− mice, and is associated with a decrease in the contribution of vasodilator prostanoids but not changes in the contribution of nitric oxide, oxidative stress or expression of All receptors.
**Testicular function and activin A in the Ins2(Akita) mouse, a model of type 1 diabetes**

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Type 1 diabetes is associated with male infertility, and has features of a chronic inflammatory condition. Male fertility is inhibited by inflammation, and activin A is an inflammatory protein that regulates testicular function. Our aim was to evaluate the role of activin A and its binding protein, follistatin, in the Ins2(Akita) mouse, which has an inactivating mutation of the insulin gene (Ins2). Mice that are heterozygous for the Ins2(Akita) mutation may or may not develop progressive diabetes.

Diabetes was determined by increased blood glucose and HbA1c. Body and testis weight were measured in diabetic and non-diabetic heterozygous Ins2(Akita) mice and wild-type mice at 12 and 24 weeks of age. qPCR was used to evaluate expression of the Inhba gene, encoding the activin A subunit, as well as follistatin, activin receptor subunits and key inflammatory cytokines (TNF, IL-1α, IL-1β, IL-8, INF-γ), in the testis. Activin A protein was detected by immunohistochemistry. Compared with mice that did not develop diabetes, diabetic mice showed a reduction of body weight at 12 and 24 weeks and a 30% reduction in testis weight at 24 weeks, indicating progressive testicular failure; however, testes morphology appeared normal. Testicular Inhba mRNA was significantly up-regulated (400-fold) only in diabetic mice at 12 weeks and returned to baseline at 24 weeks; however, immuno-localisation of activin A in Sertoli cells, interstitial macrophages and peritubular cells showed no changes at either time-point. Testicular follistatin, activin receptor subunits and inflammatory cytokine mRNA expression were not significantly altered by diabetes.

These data indicate that reduced insulin, leading to the development of diabetes in Ins2(Akita) mice, results in reduced testicular function, without evidence of inflammation. Activin A is implicated in this loss of function, because of the direct correlation between elevated activin A at 12 weeks, the development of diabetes and testicular damage.

**Ovarian function is regulated by the mineralocorticoid receptor**

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The role of the mineralocorticoid receptor (MR) in salt and water balance is well established. What remains less clear is its role in non-classical tissues. Our interest in the role of the MR in ovarian biology led us to establish a granulosa cell specific MR-null mouse line. We have previously described the abnormal phenotype of the KO ovaries observed in these mice: diffuse stroma, degenerate oocytes and vacuolated/disorganised follicles, theca and corpora lutea. The aim of these studies was to investigate the expression of proteins and genes by the ovary which may be regulated by the MR. The granulosa cell specific MR-null mouse was generated by crossing AMHR2cre mice (provided by Prof M. Matzuk, BCM) with our MR floxed mice. Ovaries were collected from null mice and littermate controls at 7–10 weeks of age for immunohistochemical and gene expression analyses. Formalin-fixed ovarian sections were immunostained for the MR, progesterone receptor (PR) and 11β-hydroxy steroid dehydrogenase 2 (11β-HSD2). RNA was extracted from whole ovaries and prepared for gene expression analysis using the Fluidigm Biomark™ HD system with Taqman primers. MR immunohistochemistry was used primarily to confirm genotyping results; these analyses did not always correspond, with some null ovaries having detectable protein in their granulosa cells (GC). Both the PR and 11β-HSD2 proteins were localised to GC and some stromal cells in control and null ovaries; only the intensity of the 11β-HSD2 protein appeared reduced. Our preliminary Fluidigm analysis assessed the expression of Cox2, Wnt4, Rank-ligand, Id4, SCC, ERα, Sgk1, TGFβ1 and the PRL receptor, along with appropriate housekeeping genes. Sgk1, Rank-ligand and TGFβ1 levels were increased in null mouse ovaries relative to controls whilst the other genes were unchanged. These results further support our hypothesis that the MR plays a central role in folliculogenesis.

**The generation of bioactive inhibins in the absence of activins**

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Inhibins are negative regulators of pituitary FSH production, and instrumental in the regulation of ovarian and testicular gamete production. The bioactivities of inhibins are dependent on the opposing actions of structurally related activins. Inhibins are synthesised as heterodimers of α- and β-subunits, each comprising an N-terminal pro- and C-terminal mature domain. Following dimerisation, the inhibin α- and β-subunit prodomains are enzymatically cleaved from the mature domains to enable ligand bioactivity. Activins are formed as homodimers of two β-subunits and consequently, in every cell that synthesises inhibin there is a concomitant production of activins. This naturally occurring simultaneous production of inhibins/activins has greatly impacted the study of inhibins in the isolation of activins. In this study, we sought to develop a method to enable the production of bioactive inhibins in the absence of activins. Initially, we used site-directed mutagenesis to enhance processing of the inhibin α- and βA/B-precurors. Improved cleavage of the inhibin precursors resulted in increased production of mature inhibin and...
activin forms relative to unmutated controls. Proportionally, inhibin production relative to activin was greatest where cleavage was enhanced only in the α-subunit. Additionally it was found that the ratio of inhibin to activin production in HEK293F cells could be increased by as much as 300-fold using a 4 to 1 α:β-subunit transfection ratio. To eliminate contaminating activin we incorporated an inactivating mutation into the βα-subunit, Met418→Ala. Although disruptive for activin bioactivity, here we show that the M418A mutation is dispensable for inhibin in vitro activity using an LβT2 gonadotroph assay. In combination, enhancement of inhibin precursor processing and incorporation of the M418A mutation, favoured the production of bioactive inhibins in isolation of activins. This approach will facilitate ongoing studies examining the independent biological roles of inhibins.

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Extracellular vesicle-mediated growth in androgen-deprived prostate cancer cells

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Unraveling an identity for the androgen receptor-expressing mammary epithelial cell

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Introduction: Androgens inhibit normal breast growth, while both androgen receptor (AR) agonists and antagonists are being trialled in women with different subtypes of breast cancer, including estrogen receptor (ERa)-positive and ERα-negative. However, AR signaling exhibits context-dependent activity among breast cancers1. We hypothesize that these disparities reflect differences in AR expression or action in the normal breast. Therefore, we undertook an in situ investigation of AR expression in relation to expression of established markers of mammary epithelial cell (MEC) proliferation and differentiation in normal human breast, and associated this with the presence of an adjacent benign or malignant ERα-positive lesion.

Methods: Confocal immunofluorescence was employed to associate expression of AR in normal MECs with markers of proliferation (Ki67) and differentiation (basal - P63, SCF; alveolar – KIT, ELF5; luminal hormone-sensing - ERa, progesterone receptor (PR)). A separate assessment was made of the relationship between the expression of AR, ERα, PR and markers of AR activity (PSA, apolipoprotein D) in normal breast tissue adjacent to benign or malignant ERα-positive lesions, to associate androgen responsiveness with progression of breast cancer.

Results: High AR expression in normal MECs associated with hormone-sensing cells expressing ERα and/or PR, and were largely negative for alveolar, luminal progenitor and basal markers. A small proportion of AR-expressing MECs co-expressed the alveolar marker KIT, illustrating the potential for AR signalling to play roles in regulating the development or function of multiple MEC lineages. The expression of AR-responsive genes was reduced in normal MECs adjacent to malignant versus benign breast lesions.

Conclusions: AR expression in normal human breast was associated with the hormone-sensing lineage and an inactive state of growth and differentiation. That expression of AR-responsive genes is decreased in tissue adjacent to malignant lesions supports the use of AR agonists as targeted therapy for ERα-positive breast cancer.


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Androgen receptor function: The biological basis of diseases linked to testosterone

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Androgen receptors located throughout the body, regulate most, but not all actions of testosterone. This ligand regulated nuclear transcription factor has clinically significant effects in almost every organ system of the body. Studies of mutations of the androgen receptor in humans and targeted tissue-specific deletion in genetically modified animal models are powerful tools for understanding the functional role and significance of the androgen receptor. Clinically relevant human mutations of the androgen receptor are linked to the motor neurone disease, disorders of sexual development and a variety of hormone dependent tumours. Removal of the ligand testosterone in humans and mice offers further valuable insight into the role and function of the androgen receptor. This review will link androgen receptor function, as understood from animal models to human function and pathology. Tissue specific deletion of the androgen receptor in genetically modified mice using the cre-lox
system will be compared to the effects of androgen deprivation in humans. Effects in muscle, bone and adipose tissue will be used to demonstrate the widespread function of the androgen receptor.

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Androgens and cardiovascular risk
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Ageing is accompanied by a reduction in circulating testosterone (T), and progressive accumulation of medical morbidities. There is ongoing debate as to whether low T contributes to ill-health, particularly to increased risk of cardiovascular disease, as opposed to being a biomarker for its presence. Despite this uncertainty, prescriptions for T are rising on a background of concern over potential adverse effects. Observational studies show lower risk of cardiovascular events in older men with higher T concentrations. In longitudinal analyses from the Western Australian Health In Men Study (HIMS) we have shown that optimal circulating T predicts survival in older men, and that higher T concentrations are independently associated with reduced incidence of stroke. Furthermore, in HIMS men with higher concentrations of the more potent androgen dihydrotestosterone (DHT) experienced lower mortality from ischaemic heart disease. Concern has been raised following the Testosterone in Older Men with Mobility Limitations (TOM) trial, which was terminated due to excess cardiovascular adverse events reported in the T treatment arm. However, no such signal was seen in a comparable study of T in intermediate-frail and frail older men. Of note, these and other randomised controlled trials (RCTs) of T supplementation have been underpowered for the outcome of cardiovascular events. Recent meta-analyses generally have not shown an excess of cardiovascular adverse events to be associated with T therapy. Retrospective studies of prescription databases have produced controversial and conflicting results. Thus additional RCTs are required to clarify the role of T supplementation to modulate cardiovascular risk in older men in the absence of pituitary or testicular disease. T replacement therapy should be considered in androgen deficient men, with evaluation of potential benefits and risks.

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Andropause/Low T: The Masquerade of Sick Eugonadism
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Awareness of mortality often leads to wishful thinking about delaying or reversing ageing. With sufficient prosperity, this flourishes into a fashionable hobby which had its zenith in the era of rejuvenation quackery at the turn of the 20th century involving testis extracts, slices or manipulation. This fad vanished in the 1930’s with the discovery of testosterone (T) during the Depression but resurfaced in the last two decades under the guise of “andropause”, “late onset hypogonadism” and “LowT”. Over-interpreting observational studies linking cardiovascular disease and other disorders with low circulating T to assume protective effects of T has led to massive increases in T prescribing. However in men without reproductive system pathology, low circulating T associated with systemic disorders represents a “sick eugonadal” or “non-reproductive illness syndrome” rather than a deficiency state as part of an adaptive hypothalamic response to non-reproductive disorders. As these effects may be beneficial, neutral or harmful, T treatment for non-reproductive system disorders requires rigorous proof of efficacy and safety. In addition, recent studies also provide troubling, albeit inconclusive, adverse cardiovascular safety signals for T treatment of men who have no reproductive system disorders.

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Using translating ribosome affinity purification (TRAP) to investigate gene expression in beige or ‘browned’ adipocytes
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Background: Brown adipose tissue (BAT) is a specialized organ that dissipates chemical energy to protect against hypothermia and obesity through nonshivering thermogenesis. BAT has been identified in humans, and its activation may reduce body weight and improve glucose homeostasis. Independently of BAT, thermogenic fat cells (called ‘beige’ adipocytes) have also been identified in depots of white adipose tissue (WAT). This browning of WAT occurs following cold exposure, chronic sympathetic stimulation, or thiazolidinedione treatment. In mice, we have previously reported that iron chelation causes browning of WAT, concomitantly increasing energy expenditure and attenuating high-fat diet (HFD)-induced weight gain.

WAT is highly heterogeneous, being composed of adipocytes, preadipocytes, vascular endothelial cells, and immune cells. In a WAT depot, therefore, it is impossible to determine changes in gene expression specifically in beige adipocytes. We have used translating ribosome affinity purification (TRAP) to purify ribosomal RNA from genetically-defined beige adipocytes within WAT.
Methods: TRAP mice are transgenic for a rosa26-lox-stop-lox-EGFP-ribosome fusion construct. When crossed with mice expressing Cre, the ‘stop’ is excised, and the ribosomes of Cre-expressing cells are labeled with EGFP. RNA is then isolated using a GFP antibody. TRAP mice were crossed with Prdm16-Cre mice, to generate Prdm16-TRAP mice, which express Cre specifically in beige adipocytes (from Bruce Spiegelman). These mice were fed HFD+siron chelator (30 mg/kg/day) for two weeks. Beige adipocyte RNA was isolated from inguinal subcutaneous WAT using TRAP.

Results: Iron chelation increased the expression of Ucp1 mRNA >80-fold, and expression levels of other regulators of thermogenesis (Ppargc1a, Prdm16, and Cidea) were increased 8 to 24-fold vs. untreated mice. Leptin (Lepr), a marker of WAT, was reduced 15-fold following iron chelation treatment, and expression of the beige adipocyte marker Tbx1 was increased 8-fold.

Conclusions: TRAP is a valuable molecular tool for studying gene expression changes specifically in beige adipocytes.

Expression of hexosamine signaling pathway genes in placentae from women with gestational diabetes mellitus (GDM)

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Background

The hexosamine signaling pathway (HSP) leads to the posttranslational addition of O-linked N-acetylglucosamine (O-GlcNAc) to proteins, altering their fate and function. Fructose-6-phosphate is funneled from the glycolytic pathway into the HSP by glutamine:fructose-6-phosphate aminotransferase (GFAT1). O-GlcNAc transferase (OGT1) adds O-GlcNAc and the O-GlcNAcase OGA removes it. GFAT1 acts as a nutrient sensor and its activity is dependent on glucose and amino acid metabolism. In type 2 diabetes mellitus, GFAT1 mRNA levels and activity are increased in skeletal muscle. O-GlcNAc levels are associated with the development of insulin resistance. This study aims to analyze placental expression of important enzymes in the HSP in GDM.

Methods

mRNA was extracted from placentas from 10 women with and 30 women without GDM matched for BMI, gestational age at delivery and birthweight. Expression of GFAT1, OGT1 and OGA was assessed by qPCR using the geometric mean of expression of TBP and B-Actin as endogenous controls. Non-parametric methods were used to compare expression between the groups. Immunohistochemical staining for GFAT1 was performed on five GDM and five control placental samples.

Results

Placental mRNA expression of GFAT1 was higher in women with GDM (2.16 (1.21-6.78) median (IQR) AU) than in women without (0.76 (0.48-2.25), P<0.05). OGT1 expression also was higher in women with GDM (2.53 (0.89-8.18) vs. 0.49 (0.16-2.87), P<0.05). There was no difference in the expression of OGA. The expression of these genes was not correlated with maternal BMI or infant birth weight. Immunohistochemical staining demonstrated preferential staining of the placental spongiotrophoblast and endothelial cells.

Conclusion

Maternal GDM is associated with an increase in the placental expression of two key enzymes in the HSP. The direction of change is suggestive of a funneling of proteins toward the HSP and increased O-GlcNAc cycling. These changes are not associated with changes in infant birth weight.

Beta Adrenergic receptors stimulation attenuates hyperglycemia-induced inflammation and apoptosis via NF-κB and IκBα in endothelial cells

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Background: Apoptosis and inflammation are important features of endothelial dysfunction in diabetes. NF-κB plays a key role in inflammation and apoptosis through its ability to induce transcription of pro-inflammatory genes. In this study, we investigated the effect of β-adrenergic receptor stimulation on NF-κB and IκBα mediated apoptosis, inflammatory cytokines and adhesion molecules in hyperglycaemic HUVECs.

Methods: Human umbilical vein endothelial cells (HUVECs) were cultured in high (25 mM) and low (5 mM) concentrations of glucose. Cells were treated with 5, 10 and 20 μM isoproterenol and propranolol for 6, 12 and 24 hours. The experimental procedures consisted of Flow Cytometry, Western Blotting, ELISA, LDH release, DCFH-DA and TUNEL assays.

Results: Beta-adrenergic receptors stimulation by isoproterenol significantly reduced the levels of TNF-α, IL-1b, IL-6 and IL-8. TNF-α induced expression of ICAM-1, VCAM-1 and E-selectin were significantly reduced when treated with beta-ARs agonist. Significant dephosphorylation was observed at Ser-536 of NF-κB and Ser-32 and Ser-36 of IκBα in beta-ARs agonists treated HUVECs. Isoproterenol also significantly reduced apoptosis and ROS generation. No effect was observed on cell cycle arrest and Tyr-42 phosphorylation of IκBα upon isoproterenol treatment. The effect of isoproterenol was reversed by the antagonist propranolol.
Conclusion: Our data demonstrate that beta adrenergic receptors stimulation has protective effect on HUVECs. Stimulation of β-adrenergic receptor induces these changes via NF-κB and IκBα.

Age-related changes in estradiol and longitudinal associations with fat mass in men

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Context: In men, circulating 17β-estradiol (E2) originates primarily from aromatization of testosterone (T) in peripheral tissues, particularly adipose tissue. The effect of ageing and obesity on circulating E2 remains unclear.

Objective: To investigate 5-year changes in serum E2 and the association with T and fat mass in Australian men.

Design: Participants were 725 community-dwelling men, aged 35 years and older, without established disease of, or medications affecting, the hypothalamus-pituitary-gonadal axis. At baseline and 5-year follow-up, socio-demographic and health-related data including behaviours, chronic conditions, and medication use were collected by questionnaire. E2 and T were assayed by liquid chromatography-tandem mass spectrometry and Sex hormone-binding globulin (SHBG) by immunofluorescence assay.

Separately, we determined the effect of 28 days over-feeding a high fat energy dense diet on adipose tissue aromatase mRNA measured by qPCR in 8 male volunteers (mean age 35.4 ± 7.8 years, BMI 26.1 ± 3.8 kg/m2).

Anthropometry and fat mass were assessed clinically and by dual-energy X-ray absorptiometry respectively, in both studies.

Results: At baseline, mean age was 53.0 ± 10.8 years. Mean serum E2 levels at baseline and follow-up were 94.9 ± 34.8 and 89.4 ± 30.4 pmol/L respectively (-1.1 pmol/L/year). On multivariate analyses, E2 change was associated with T change (p<0.001) but not age or percentage total fat mass. Changes in T and T/E2 ratio were inversely associated with change in fat mass (p=0.003 and 0.012 respectively). The change in T/E2 was consistent across fat mass quartiles.

Overfeeding increased fat mass but not aromatase mRNA expression in abdominal subcutaneous fat.

Conclusion: Circulating E2 levels are primarily dependent on T. With increasing fat mass, E2 decreases less than T, likely due to the greater overall aromatase activity despite no increase in aromatase expression.

Metabolic and fetal benefits of endurance exercise training for females born small on high fat diet

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Intrauterine growth restriction programs adult metabolic disease which is exacerbated with “second hits” such as pregnancy and overweight/obesity in females born small. We have recently reported that the physiological challenge of pregnancy unmasks glucose intolerance in females born small. This study determined if the known adverse physiological adaptations to pregnancy in rats born small are exacerbated with a high fat diet (HFD) and whether endurance exercise training can prevent these complications.

Uteroplacental insufficiency was induced by bilateral uterine artery ligation (Restricted) or sham (Control) surgery on E18 in Wistar-Kyoto rats. Female offspring were fed a chow or HFD (43% kcals from fat) from 5 weeks of age to mating (20 weeks) and throughout pregnancy. Female rats were exercised on a treadmill 4 weeks before mating and throughout pregnancy. Glucose tolerance test was performed (E18) and dorsal fat weights and plasma leptin concentrations were measured at E20.

Restricted and Control female rats that were exposed to a HFD were heavier with ~30% more dorsal fat than females on a chow diet. Exercise prevented dorsal fat gain in Restricted HFD compared to sedentary HFD Restricted rats. Similarly, plasma leptin concentrations were 59% higher in Restricted and 30% higher in Control female rats on a HFD compared to females on chow diet. HFD exacerbated the pre-existing glucose intolerance (+15% area under curve) in pregnant females born small compared to growth-restricted females on a Chow diet and exercise prevented the development of glucose intolerance. Exercise-training prevented the reduced fetal weight in females born small, despite no effect of exercise on placenta weight.

We demonstrated that females born small are at a greater risk of increased adiposity and exacerbated glucose intolerance when exposed to a HFD and these were prevented by the lifestyle intervention of exercise.
Effect of low dose glucocorticoid therapy on arginine metabolism in patients with rheumatoid arthritis

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Background: Low dose prednisolone therapy is associated with better endothelial function in patients with rheumatoid arthritis (1). This contrasts findings in hypopituitary patients, where an increase in glucocorticoid dose impaired endothelial function (2). In the endothelium arginine is converted by nitric oxide synthase to citrulline and nitric oxide, a potent vasodilator. However, arginine can also be converted to ornithine or homoarginine, reducing its availability. Furthermore, the arginine metabolites asymmetric dimethyl arginine (ADMA), N-mono methylated arginine (MMA) and symmetric dimethyl arginine (SDMA) inhibit nitric oxide synthase directly or indirectly and are associated with increased cardiovascular risk. We hypothesized that rheumatoid arthritis causes specific changes in arginine metabolism that influence the response to glucocorticoids.

Methods: Eighteen patients with rheumatoid arthritis who had not taken prednisolone for > 6 months (non-GC users), 18 patients taking continuous oral prednisolone (6.5±1.8 mg/day) for > 6 months (GC users) and 20 healthy controls were studied. Fasting serum concentrations of 7 key components of arginine metabolism (arginine, homoarginine, citrulline, ornithine, ADMA, MMA and SDMA) were measured by ultra-performance liquid-chromatography.

Results: There were no significant differences in age, sex and glomerular filtration rate between the groups (Table). Non-GC users had higher arginine (p=0.001), citrulline (p=0.002), ADMA (p=0.004) and MMA (p<0.001) than controls, with no significant difference in ornithine, homoarginine and SDMA (Table). ADMA (p=0.03) and SDMA (p=0.03) were lower in GC users than non-GC users, with no significant differences in other arginine metabolites between these two groups (Table).

Conclusions: Rheumatoid arthritis per se is associated with changes in arginine metabolism, including an increase in ADMA. Long term prednisolone treatment in rheumatoid arthritis is associated with lower levels of ADMA. The latter might account, at least partly, for the improved endothelial function observed in these patients.


Thrombospondin-1 is a glucocorticoid responsive protein and potential biomarker of glucocorticoid activity.

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Introduction: Glucocorticoids are widely prescribed medications, but supraphysiological doses are associated with increased morbidity and mortality, and under-dosing is also potentially harmful in adrenal insufficiency. Dose optimisation would be greatly enhanced by the availability of a biomarker of glucocorticoid activity. Thrombospondin-1 (TSP-1) is a matricellular protein which is upregulated by glucocorticoids in several in vitro systems. The aim of the study was to determine if TSP-1 is altered by acute and chronic states of glucocorticoid excess and deficiency in human subjects.

Methods: Three studies have been undertaken: (i) A cross-sectional study compared morning plasma TSP-1 in 20 healthy volunteers, 6 patients with Cushings’s syndrome and 16 patients with secondary adrenal insufficiency; (ii) An acute interventional study assessed the effects of a single 4 mg dose of dexamethasone after 8, 12 and 16 hours on peripheral blood mononuclear cell (PBMC) TSP-1 mRNA levels and plasma TSP-1 in 20 healthy volunteers; (iii) A short term interventional study assessed the effect on plasma TSP-1 of increasing the hydrocortisone replacement dose from ≤20 mg/day to 30 mg/day for 7 days in 16 patients with secondary adrenal insufficiency.
Results: (i) Median (interquartile range) plasma TSP-1 was lower in patients with secondary adrenal insufficiency: 139 (86-199) ng/mL, (P<0.0001) and higher in Cushing’s syndrome: 606 (497-740) ng/mL, (P<0.001) than in the healthy volunteers: 272 (237-336) ng/mL. (ii) 4 mg dexamethasone significantly increased PBMC TSP-1 mRNA levels (P<0.0001) and plasma TSP-1 concentrations (P<0.0001) in healthy volunteers, peaking at 12 hours. (iii) The higher hydrocortisone dose increased median plasma TSP-1 from 139 (86-199) to 256 (133-516) ng/mL in patients with secondary adrenal insufficiency (P<0.01).

Conclusion: TSP-1 is a glucocorticoid responsive protein, which shows promise as a biomarker of glucocorticoid activity.

The role of a day 5 metyrapone test in assessing the HPA axis post pituitary surgery, a prospective trial

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Introduction: Pituitary surgery may result in new deficits in hypothalamic-pituitary-adrenal (HPA) axis function, but protocols for administering post-operative glucocorticoids and assessing post-operative function differ widely. The objective of this prospective trial was to compare the performance of a Day 5 metyrapone test, postoperative morning cortisol levels, delayed metyrapone, short Synacthen test (SST) and insulin tolerance test (ITT) at 6-7 weeks post pituitary surgery as predictors of glucocorticoid replacement at 6 months.

Methods: The cohort consisted of 33 participants (16 women, 17 men), who had undergone 30 trans-sphenoidal surgeries and 3 craniotomies - 24 non-functioning macroadenomas, 1 meningoma, 3 Rathke’s cysts, 4 GH-secreting macroadenomas, 1 PRL-secreting macroadenoma, 1 craniopharyngioma and 1 adenohypophysis.

Morning cortisol (before 0900h) levels taken day 3 and 4 postoperatively (normal response: defined as >400nmol/L), metyrapone testing (30mg/kg) on day 5 and week 6 (normal response: 11 deoxycortic >200nmol/L), SST week 6 and an ITT week 7 (normal response: cortisol >500nmol/L for both). Post-operative glucocorticoid replacement was administered strictly per protocol. If morning cortisol was <400nmol/L and/or 11 deoxycortic <200 nmol/L after metyrapone at day 5, hydrocortisone was given at <20mg daily until later testing.

Results: Mean tumour maximal diameter was 23mm (range 3mm-49mm). The prevalence of glucocorticoid requirement at 6 months was 55%. The table illustrates sensitivity and specificity of each test as predictors of glucocorticoid replacement at 6 months.

Conclusions: These data suggest that both morning cortisol and day 5 metyrapone testing have good sensitivity in predicting glucocorticoid replacement at 6 months. However, the sensitivities of morning cortisol, metyrapone testing at both 5 days and 6 weeks and SST were all lower than ITT. Interestingly in our study, the “gold standard” ITT had low specificity for predicting glucocorticoid replacement at 6 months.

Table 1: HPA axis tests as predictors of glucocorticoid replacement at 6 months

Loss-of-function germline FGFR1 mutation identified in a patient with prolactinoma

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Background: Familial pituitary tumours are thought to be rare, occurring in approximately 5% of pituitary tumour cases (Tichomirowa et al, 2011). Germline mutations in MEN1, AIP, p27 and PRKAR1A are known to be involved (Elston et al, 2009), however recently SDHx and GPR101 have been added to the expanding list of genes implicated in the hereditary predisposition to pituitary tumours (Gill et al, 2014; Trivellin et al, 2014). Utilising next generation sequencing technology, we have developed a 300+ gene panel incorporating genes known to be involved in pituitary tumour pathogenesis, pituitary embryogenesis and broad cancer genes. We have commenced screening familial pituitary and young sporadic pituitary tumour cases with this panel. Using this approach, we identified a rare missense, heterozygous variant in fibroblast growth factor receptor 1 (FGFR1)c.485A>C; p.D162A), in a male with a childhood-onset prolactinoma whose daughter has congenital hypopituitarism. Germline mutations in FGFR1 have been implicated in congenital hypopituitarism.

Aim: To determine whether the identified FGFR1 variant p.D162A is functionally deleterious using an established culture model, in vitro.
Method: Rat L6-myoblasts which contain very low levels of endogenous FGF receptors and ligands, were transfected with wild-type and mutant FGFR1 pmv-SPORT6 expression vectors along with a luciferase reporter driven by 6 tandem repeats of the osteoblast-specific core binding sequences of the FGF responsive osteocalcin promoter (Kim et al. 2003). Cells were treated with recombinant human FGFR2 ligand and then lysed for luciferase assay 24 hours later. Treatments were conducted in triplicate and cultures repeated three times.

Results: FGFR1 (pD162A) variant exhibited a 40% reduced function (p<0.001) compared to wildtype.

Conclusion: We have identified a loss-of-function mutation in FGFR1 in a patient with a pituitary tumour. Identification of the same mutation in the daughter and in other families may also implicate FGFR1 in the hereditary predisposition to pituitary tumours.


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Growth hormone replacement improves anaerobic capacity and physical function in adults with growth hormone deficiency

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Introduction The anaerobic energy system initiates all physical activity and subserves many aspects of physical function of daily living. Anaerobic and physical capacities are reduced in GH deficient (GHD) adults (1).

Aim To investigate whether GH improves anaerobic capacity, physical function and quality of life (QoL) in GHD adults.

Method 18 GHD adults were randomized into a 1-month double-blind placebo-controlled crossover GH (0.5mg/day) study followed by a 6-month open phase. Anaerobic capacity was assessed by the Wingate test and aerobic capacity by the VO2max test. Physical function was assessed by the stair-climb test, chair-stand test and daily step count by pedometry. QoL was assessed by the AGHDA questionnaire. Between and within treatment effects were analyzed by repeated-measures ANOVA and one tailed t-test.

Results GH treatment normalized IGF-I concentration. Stair-climb and chair-stand performance increased significantly during 1-month GH and placebo treatment. Compared to placebo, GH treatment for 1 month did not affect any outcome measure (table). GH treatment for 6 months significantly increased anaerobic power, chair-stand repetitions, daily step count (p=0.07), energy and vitality QoL scores (p=0.07), but not stair-climb duration or VO2max.

Table: Changes in outcome measures after 1 and 6 months of GH replacement

<table>
<thead>
<tr>
<th>GH Treatment</th>
<th>Wingate Watts</th>
<th>VO2max L/min</th>
<th>Stair-Climb Seconds</th>
<th>Chair-stand Number</th>
<th>Pedometry Steps/d</th>
<th>QoL Scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 month</td>
<td>3.4±8.7</td>
<td>0.07±0.0</td>
<td>-2.4±1.2</td>
<td>2.2±0.8</td>
<td>-270±273</td>
<td>-3.5±1.4</td>
</tr>
<tr>
<td>6 months</td>
<td>15.4±8.7*</td>
<td>0.01±0.1</td>
<td>-0.8±0.3</td>
<td>6.1±1.2*</td>
<td>1169±659</td>
<td>-6.9±1.8</td>
</tr>
</tbody>
</table>

*p<0.05 compared to 1-month placebo

Summary 1 month of GH replacement was ineffective. 6-months replacement improved Wingate and chair-stand performance, daily step counts and QoL domains but not VO2max in GHD adults.

Conclusion GH replacement improves anaerobic capacity, physical function and QoL in a time-dependent manner in GHD adults. Improvement in anaerobic but not aerobic energy system is associated with improvement in physical function and related QoL.


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Germline mutation in the MET proto-oncogene, receptor tyrosine kinase/hepatocyte growth factor receptor (MET) in a patient with phaeochromocytoma – a new gene for this disorder

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1 University of Queensland Diamantina Institute, Translational Research Institute, Brisbane, QLD, Australia

References


Germline mutations in one of 16 identified susceptibility genes are detected in up to
behavioural evidence is lacking.

- executive function,
- complex, non
- relationship was thought to be

Context

4.

3.

2.

1.

Karen M Rothacker

individual analysis of a large population

Conclusion: These results suggest that the CAR magnitude influences cognitive performance, particularly exec
changes in cognitive performance.

Results: The magnitude of the CAR was not significantly different between the two days of testing, but was highly correlated
and memory, however functional beh

Background: The cortisol awakening response (CAR) is the glucocorticoid peak that occurs within the first hour of awakening. Existing evidence supports a relationship between the magnitude of the CAR and the neural mechanisms that underlie learning and memory, however functional behavioural evidence is lacking. The aim of this study, therefore, was to determine whether the CAR magnitude was associated with same-day cognitive performance.

Methods: Saliva was collected at 0, 15, 30 and 45 minutes after awakening in 31 healthy adults

Discussion: The magnitude of the CAR was not significantly different between the two days of testing, but was highly correlated

Conclusion: These results suggest that the CAR magnitude influences cognitive performance, particularly executive function, throughout the day. These effects do not appear to be driven by changes in perceived stress or circulating cortisol at the time of testing. Whether this increase in cognitive performance is a direct or indirect effect of the CAR is currently unknown.

Reconciling the log-linear and non-linear aspects of the TSH-free T4 relationship: intra-
individual analysis of a large population

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Context: The TSH-T4 relationship is central to thyroid pathophysiology and diagnosis of thyroid disease. Previously the relationship was thought to be inverse log-linear, but recent cross-sectional studies from our group and others report a complex, non-linear relationship (1-3). There have been no large, intrindividual studies of the TSH-T4 relationship.
Objective: To analyze the TSH-free T4 relationship within individuals.

Methods: We analyzed data from 13,379 individuals, each with 6 or more TSH/free T4 measurements and at least a 5-fold difference between individual median TSH and minimum or maximum TSH. Linear and non-linear regression models of log TSH on free T4 were fitted to data from individuals, and goodness of fit compared by likelihood ratio testing.

Results: On comparing all models, the linear model achieved best fit in 31% of individuals, followed by the quartic (27%), cubic (15%), null (12%) and quadratic (11%) models. After elimination of least favoured models (with reassignment of individuals to the best fitting, available models), the linear model fitted best in 43% of individuals, quartic in 42%, and the null model in 15%. As the number of records per individual increased, so did the proportion of individuals in whom the linear model achieved best fit, increasing to 62% of individuals with 20 or more records. When the linear model was applied to all individuals and plotted according to individual median free T4 values, differences in slope and intercept described a non-linear relationship between log TSH and free T4.

Conclusions: The log TSH-free T4 relationship appears linear in some individuals and non-linear in others, but is predominantly linear in the most informative individuals with the largest number of results. An inverse log-linear relationship within individuals can be reconciled with a non-linear relationship across a population.


Endocrinopathies associated with immune modulation therapy for the treatment of metastatic melanoma

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3. Melanoma Institute, Mater Hospital, University of Sydney, Sydney
4. Kolling Institute of Medical Research, Royal North Shore Hospital, Sydney

BACKGROUND

Immune modulator therapy has a demonstrated survival benefit in the treatment of metastatic melanoma. Monoclonal antibodies against regulatory immune checkpoints can enhance the immune activity against cancer cells. Agents include Ipilimumab, a monoclonal antibody against cytotoxic T-lymphocyte associated antigen 4 (CTLA-4), and Nivolumab, a monoclonal antibody against programmed death-1 (PD-1) receptor. As a consequence of immunomodulation, endocrine immune related adverse events (irAEs) can occur, but the incidence in combination or sequential immunotherapy has not been reported in large series.

METHODS

Patients with metastatic melanoma at the Melanoma Institute Australia, between April 2014 and May 2015 were treated with Anti-CTLA-4 (Ipilimumab) or Anti-PD-1 (Nivolumab or Pembrolizumab) therapy alone, sequentially or in combination. The incidence of endocrine irAEs was assessed with regular monitoring of pituitary and thyroid function.

RESULTS

26 (15%) patients were diagnosed with an endocrinopathy. 12 (6.9%) patients were diagnosed with hypophysitis, 1 (0.5%) with thyroid dysfunction and 2 (1.16%) with isolated hypogonadism. 9 (15.8%) in the Anti-CTLA-4 arm developed endocrinopathies, compared to 5 (5.7%) in the PD-1 arm. Combination Anti-CTLA-4 and PD1 was associated with increased endocrinopathies 18 (62.1%).

DISCUSSION

Endocrine related endocrinopathies as a result of immunotherapy are underreported, as there are few screening requirements2. Endocrine irAEs associated with Anti-CTLA4 are hypophysitis, thyroid dysfunction and primary adrenal insufficiency1. Studies suggest a 5% incidence of hypophysitis, and 0-4% incidence of thyroid dysfunction1. Endocrine irAEs occur less frequently with anti-PD1 therapy3. Combination ipilimumab and nivolumab therapy is associated with increased irAEs, particularly thyroid dysfunction4. There does not appear to be any predictors for the development of endocrinopathy. The time course appears more rapid than for autoimmune hypophysitis and thyroiditis with disease occurring in weeks. A heightened clinical suspicion and regular monitoring will prevent the development of morbidity especially adrenal crises.

Association between plasma adipocytokine concentrations and microvascular complications in patients with type 2 diabetes mellitus: a systematic review and meta-analysis of controlled cross-sectional studies

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Background: Adipocytokines have been variably associated with these complications in observational clinical studies. However, there are no comprehensive data examining the associations between adipocytokines concentrations and the presence of these complications.

Methods: A systematic review of cross-sectional studies comparing circulating adipocytokines in patients with type 2 diabetes (T2D) who were affected by at least one microvascular complication, with T2D patients without these complications. Relevant studies were retrieved from MEDLINE, EMBASE, Scopus and Cochrane databases. Study quality was evaluated using a modified Newcastle-Ottawa Quality Assessment Scale. Meta-analysis was performed using an inverse-variance model. Standardised mean differences (SMD) and 95% confidence intervals (CI) were calculated from which fixed or random effects models were applied.

Results: 554 abstracts were identified; 28 studies satisfied our inclusion/exclusion criteria. Study quality ranged from 4-10 (out of 11). Higher leptin levels were associated with the presence of microalbuminuria (SMD=0.41; 95%CI=0.14, 0.67; n=901; p=0.0003) and macroalbuminuria (SMD=0.68; 95%CI=0.30, 1.06; n=406; p=0.0004). Similarly, higher leptin levels were associated with the presence of neuropathy (SMD=0.26; 95%CI=0.07, 0.44; n=609; p=0.008). Higher adiponectin levels were associated with the presence of microalbuminuria (SMD=0.55; 95%CI=0.29, 0.81, n=274; p=0.001) and macroalbuminuria (SMD=1.37; 95%CI=0.78, 1.97, n=246; p<0.0001). In addition, higher adiponectin levels were associated with neuropathy (SMD=0.25; 95%CI=0.14, 0.36; n=1516; p=0.0001) and retinopathy (SMD=0.38; 95%CI=0.25, 0.51; n=1306; p<0.0001).

Discussion: This systematic review and meta-analysis suggests that blood leptin and adiponectin levels are higher in patients with diabetes and microvascular complications, making these adipokines potentially relevant as therapeutic targets or biomarkers of diabetic microvascular complications. Studies were limited by cross-sectional design, thus large prospective analyses are required to confirm these findings independent of other risk factors, and to determine their causality.

Effect of adrenocorticotropic hormone stimulation on the outcomes of adrenal vein sampling in primary aldosteronism

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2. Pathology, Monash Health, Clayton, Victoria, Australia
3. Radiology, Monash Health, Clayton, Victoria, Australia
4. Hudson Institute of Medical Research, Clayton, VIC, Australia

Background: Adrenal vein sampling (AVS) is crucial for differentiating between unilateral and bilateral causes of primary aldosteronism (PA) [1]. However, there is a lack of uniform agreement regarding use of adrenocorticotropic hormone (ACTH) stimulation during AVS [2]. At Monash Health, AVS has been performed both pre- and post-ACTH stimulation since 2010.

Aim: We reviewed the impact of ACTH stimulation on AVS success rates and outcomes including selectivity index (SI= Cortisol adrenal vein : cortisol peripheral vein), lateralization index (LI= aldosterone-cortisol ratio nondominant adrenal vein : aldosterone-cortisol ratio dominant adrenal vein and contralateral suppression index (CSI= aldosterone-cortisol ratio non-dominant adrenal vein : aldosterone-cortisol ratio peripheral vein).

Methods: An audit was conducted on AVS procedures performed at Monash Health between January 2010 and March 2015 inclusive. Clinical information was collected on screening aldosterone and renin concentration, AVS aldosterone and cortisol levels pre- and post-ACTH stimulation, adrenal imaging, blood pressure and antihypertensive medication. Successful cannulation was defined as SI > 2 pre-ACTH and >3 post-ACTH; successful lateralisation was defined as LI >3 pre-ACTH and > 4 post-ACTH, and supported by CSI <1.

Results:
Out of 28 AVS cases with pre-and post-ACTH data, cannulation success of the left adrenal vein was 81% (22/27) pre-ACTH and 96% (26/27) post-ACTH; and of the right adrenal vein was 60% (17/28) both pre-and post-ACTH. The improved cannulation rate was not associated with the timing of AVS. However, ACTH stimulation significantly lowered the LI and incorrectly obscured lateralization in five cases. These patients were diagnosed with unilateral aldosterone excess based on their pre-ACTH LI and CSI. ACTH did not significantly affect the CSI. Four of these patients have had successful surgery with one awaiting surgery.

**Conclusion:**
The rate of successful cannulation in AVS increased after ACTH stimulation, but at the cost of masked lateralization.


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**Ligand-independent activation of FGFR2c leads to XY sex reversal in humans and mice**

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**Patients with 46,XY gonadal dysgenesis (GD) exhibit genital anomalies, which range from hypospadias to complete male-to-female sex reversal. A molecular diagnosis is made in only 30% of cases. Our study identifies FGFR2c as a novel 46,XY GD locus. Human FGFR2 mutations cause various craniosynostosis syndromes including Crouzon, Pfeiffer, and Apert syndrome. Here, we describe a patient whose features we would suggest represent a new syndrome, craniosynostosis with XY male-to-female sex reversal or CSR. The patient was chromosomally XY, but presented as a phenotypic female due to complete GD, and was also diagnosed with Crouzon-like syndrome. DNA sequencing identified the FGFR2 heterozygous missense mutation, c.1025G>C (p.C342S), affecting the 2c splice isoform. Substitution of C342 by S or other amino acids (R/F/W/Y) occurs frequently in Crouzon and Pfeiffer syndrome leading to ligand-independent receptor activation. We show that the ‘knock-in’ Crouzon mouse model Fgfr2cC342Y/C342Y carrying a C342Y substitution displays variable XY gonadal sex reversal. This suggests that the C342 substitution contributed to XY sex reversal in the patient. Despite Fgfr2cC342Y being widely considered a gain-of-function mutation, the gonadal abnormalities in XY Fgfr2cC342Y/C342Y mice phenocopy those observed in Fgfr2 knockout mice. We demonstrate that sex reversal in XY Fgfr2cC342Y/- mice is rescued by wildtype Fgfr2 in Fgfr2cC342Y/- mice. This implies that ligand-independent signaling by Fgfr2cC342Y displays qualitatively different biological activities to wildtype Fgfr2c, resulting in reduced ability to promote testsis development. In conclusion, our study identifies the first FGFR2 mutation in 46,XY GD. Diagnosis of 46,XY GD should be widened to encompass FGF-signaling components.**

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**Fast weight loss does not reduce muscle strength or bone mineral density compared with slow weight loss in obese post-menopausal women**

Radhika Seimon\(^1\), Jarron Dodds\(^1\), Alice Gibson\(^1\), Jackie Center\(^2\), Tania Markovic\(^3\), Sally Mc Clintock\(^1\), Janet Franklin\(^4\), Neil King\(^4\), Ian Caterson\(^5\), Nuala Byrne\(^6\), Amanda Sainsbury\(^7\)

\(^1\)University of Sydney, Camperdown, NSW, Australia
\(^2\)Garvin Institute of Medical Research, Sydney
\(^3\)Royal Prince Alfred Hospital, Camperdown
\(^4\)Queensland University of Technology, Brisbane
\(^5\)Bond University, Brisbane

The prevalence of obesity is increasing yearly, and diet-induced weight loss is the primary treatment option. Clinicians treating obesity may hesitate to use very-low energy diets due to potential adverse effects such as reduced lean mass that could reduce muscle strength, and reduced bone mineral density (BMD). In a recent meta-analysis we found a loss of hip BMD in response to diet-induced weight loss in overweight/obese individuals.1. We therefore compared the short-term effects of fast versus slow weight loss on muscle strength and BMD in a randomised controlled trial.

This preliminary analysis included 31 obese post-menopausal women (BMI 34.0±2.5 (SD) kg/m2, age 56.8±4.1 years). Participants were randomised to either 16 weeks of FAST or SLOW weight loss (70% or 30% energy restriction, respectively). To help preserve lean mass, protein supplement was added to the VLED so both diets had a protein intake of 1g/kg body weight per day. Body weight, muscle strength (JAMAR hand dynamometer), total hip and spine BMD (Hologic Discovery Dual-energy X-ray absorptiometry) were measured at 0 (baseline) and 16 weeks after commencing energy restriction.
The FAST group lost more weight than the SLOW group (FAST: 18.9±4.0%, SLOW: 7.1±3.1% of baseline body weight; P<0.001). Compared to baseline, there was no short-term effect of either diet on muscle strength (FAST: 2.9±12.0%; SLOW: 1.1±12.0%) or BMD (Hip: FAST: -1.5±4.0%; SLOW: 0.0±3.7%; Spine: FAST: -1.5±2.8%; SLOW: -1.1±2.8%), and no difference in muscle strength (P=0.7) or BMD (Hip: P=0.3; Spine: P=0.7) between groups. These preliminary findings suggest there is no short-term adverse effect of fast or slow weight loss on muscle strength or BMD when protein intake is adequate, despite fast weight loss inducing a 2.5-fold greater weight loss. In terms of muscle strength and bone density, fast weight loss with adequate protein intake is thus a valid obesity treatment option.


A/Prof Simon Barry, University of Adelaide

Simon Barry

1.CYWHS, North Adelaide, SA, Australia

Research Interests:

My lab is interested in how a healthy immune system balances being ready to react by swiftly fighting off pathogens, while maintaining tolerance to harmless challenges such as food and body tissues. My lab is focused on the molecular basis of function and fitness in human regulatory T cells, and the role of the transcription factor FOXP3 in orchestrating this. There is increasing evidence that in a wide number of disease states including autoimmune diseases such as Type 1 diabetes and IBD, Treg cells fail to regulate the immune system effectively, and allow inappropriate destruction of tissues that are essential for life. In order to understand how this breaks down in disease one must first understand what is the basis of a healthy Treg. To do this we use a number of state of the art gene discovery tools such as microarrays and next generation sequencing to identify and then confirm the key genes in Treg function. We were the first to identify the targets of FOXP3 in human Treg using chromatin immunoprecipitation, and we are now developing systems biology approaches to modeling the gene regulation networks in human Treg from healthy and disease samples. Tight regulation of target genes including SATB1 by FOXP3 and microRNAs is a key mechanism by which Treg retain their phenotype, and breakdown of this results in loss of function. Most recently we have established chromatin conformation capture in order to fully map the regulatory interactions inhuman Treg. A key goal of our research is to fully map the defects resulting in loss of Treg function in disease, so that new approaches to preventing or reversing this can be developed.

Training:

After a B.Sc (hons) at Kings College London and Ph.D at Mill Hill, London, Dr Barry undertook postdoctoral training in Adelaide and at the University of Washington in Seattle. He then spent 4 years working as a discovery scientist at Immunex and AMGEN in the US, prior to returning to Adelaide to set up a lab.

Dr Leigh Guerin, Phillips Ormonde Fitzpatrick

Leigh Guerin

1.Philips Ormonde Fitzpatrick, Adelaide, SA, Australia

Leigh has a Bachelor in Medical and Pharmaceutical Biotechnology, a Graduate Certificate in Science and Technology Commercialisation, a PhD in Medicine and a Masters in Intellectual Property Law.

As an honours and PhD student, Leigh worked in the field of reproductive immunology specialising in mechanisms of immune tolerance. Having completed his PhD at the Adelaide University, Leigh travelled to Boston and took up a postdoctoral research position in the Department for Stem Cell & Regenerative Biology at Harvard University where he studied lipid antigen presentation by macrophages.

Having always had an interest in the commercialization of research, and the translation of basic science into practical outcomes, Leigh took an opportunity in 2011 to move from research and into the field of intellectual property whilst at the same time moving back home to Adelaide. Leigh now works as a Patent Attorney specializing in biotechnology and works with clients to identify and protect their inventions.

Dr Melanie Bagg, The Australian Science Media Centre

Melanie Bagg

1.The Australian Science Media Centre, Adelaide, SA, Australia

Melanie has a PhD in Medicine, Obstetrics and Gynaecology, from the University of Adelaide, has completed part of the MBA program at University of Adelaide and more than 10 years of experience in science communication, marketing management and education outreach. Melanie has joined the team from the Faculty of Sciences, University of Adelaide where she helped
create and was inaugural editor of e-Science magazine, a unique digital science publication with international reach. Whilst at the University, she also spent over 5 years working closely with journalists at the SA Advertiser on specialist science print publications and a weekly “Can you believe it?” Column. Melanie is extremely passionate about science communication and outreach, in particular the way science is presented to the public via the media.

Ryan Rose, Fertility SA

Ryan Rose

1. FertilitySA, Adelaide, SA, Australia

Ryan Rose an embryologist/researcher at FertilitySA studied a PhD in Obstetrics and Gynaecology at The Robinson Research Institute in The University of Adelaide. His research was heavily focussed on basic oocyte biology and determining mechanisms of oocyte maturity. During his PhD, Ryan began casual work at Fertility SA and upon completion of his research obtained a full time position in FertilitySA’s laboratory. Primarily, Ryan works in routine IVP however he has major roles in further developing research at Fertility SA and encouraging active participation by embryologists and staff. Since his appointment he has been involved in the preparation of multiple clinical trials, research projects and translational work from bench to clinic. Although stepping away from academia, Ryan still maintains strong relationships with multiple universities and has built strong collaborations between FertilitySA and researchers across Australia.

Kiri Beilby, Origio

Kiri Beilby

1. Origio, Måløv, Denmark

Kiri is a graduate from the University of Sydney, whose Ph.D. described the function and fertility of spermatozoa that had been sex-sorted for use in animal breeding programs. She has worked at the University of Sheffield studying the maternal-gamete interactions between sperm and the reproductive tract; and within the School of Veterinary Medicine in Hannover, quantifying the gene expression of in vitro and in vivo produced embryos. Following a casual lectureship in reproductive endocrinology at the University of Sydney, Kiri completed a graduate diploma in science communication at the Australian National University. She joined ORIGIO Australasia as a product consultant and account manager in 2010, and in 2013 moved to Copenhagen to work as the international product manager of culture systems for ORIGIO a/s. Her aim today is advancing the performance and usability of commercial embryo culture media, through strong communication between global markets and industry.
Effects of dietary probiotic on growth performance, blood characteristics, and immune responses to a lipopolysaccharide challenge of Hanwoo heifers

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Our objective of the study was to effect of probiotics on the immune response of Hanwoo heifers. Lipopolysaccharide (LPS) challenge was used for investigating physiological response of dietary probiotics. A completely random design was used (4 pens; 2 pens/treatment; 5 heifers/pen). After the cattle fed probiotic for 5 months, 16 heifers were transported and acclimated to environmentally controlled chambers. Heifers were fitted with indwelling jugular catheters prior to 24 hours of the LPS challenge. Blood samples were collected at 30-min intervals from -1 to 6 h (0 h; 1 μg/kg BW of LPS from Escherichia coli O111:B4). Glucose, non-esterified fatty acid (NEFA), albumin, triglyceride, total protein, phosphorus concentrations, plasma CBC (WBC, RBC, Platelet, Neutrophils, Lymphocytes, Eosinophils, Basophil, Hemoglobin), and pro-inflammatory cytokines (TNFα, IL6, IL1b) were determined from blood samples. Response to the LPS challenge over time was analyzed by ANOVA with the MIXED procedure of SAS. Overall ADG and serum compositions did not differ between probiotic or control diet for 5 months (P >0.05). Pre-LPS NEFA concentration did not differ (P >0.05), but probiotic treated heifers was decreased at 2 hours after LPS challenge. NEFA concentration was decreased at 2 hours after LPS challenge in probiotic treated group (P <0.05). Serum triglyceride was peaked at 0.5 h after LPS challenge in of probiotic treated heifers (P <0.05). There was no difference at CBC test between treatment pre- and post - LPS challenge except red blood cell (RBC). Plasma RBC concentration was increased from 0.5h to 3h post-LPS challenge in probiotic treated heifers. These data suggest that probiotic diet did not directly altered immune response to Hanwoo heifers but indirectly regulated lipid metabolism of Hanwoo heifers at the LPS challenge.

Endocrine collateral damage

Amy Hsieh1, Greg Hockings1, Elisabeth Nye1,2

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Monoclonal antibodies such as Ipilimumab (against cytotoxic T-lymphocyte-associated antigen 4 [CTLA-4]) and nivolumab (against programmed death protein 1 [PD-1]), block inhibitory regulatory T cell molecules and achieve anti-tumour effect by enhanced T cell activation at the cost of autoimmunity. We report a case of presumed autoimmune hypophysitis and type 1 diabetes after treatment with ipilimumab and nivolumab for metastatic melanoma. A 54-year-old woman presented with seizures and confusion. Medical history included melanoma with intracranial metastases treated with craniotomy, radiation and a course of ipilimumab nine weeks prior. MRI excluded new cerebral lesions but showed an enlarged pituitary not present previously. Static anterior pituitary function evaluation revealed hypopituitarism involving the pituitary-thyroid and pituitary-gonadal axes (T4 of 6.2 pmol/L with TSH of 1.2 mIU/L; low gonadotrophins of FSH 8 IU/L and LH 1 IU/L). ACTH insufficiency was suspected but could not be established due to concurrent dexamethasone therapy (cortisol < 35 nmol/L, ACTH < 5 ng/L). A clinical diagnosis of ipilimumab-induced hypophysitis (IH) was made. Despite complications, the patient completed the ipilimumab course and then received nivolumab. Five weeks later, she presented with severe symptomatic hyperglycaemia (serum glucose 21.7 mmol/L) and ketoacidosis (pH 6.91, serum beta-hydroxybutyrate 9.4 mmol/L), requiring an insulin infusion. Abdominal CT showed a normal pancreas with no radiological evidence of pancreatitis or metastasis. The acute presentation with hyperglycaemia, ketoacidosis and low C-peptide levels led to the diagnosis of presumed autoimmune diabetes. Serum autoantibodies (IA2 and GAD65) were negative. There is little data on nivolumab-induced autoimmune diabetes. It has been reported in one recent study. This is the first report of ipilimumab-induced hypophysitis followed by apparent nivolumab-induced type 1 diabetes. These are uncommon adverse events of immunotherapy but are expected to rise in incidence as immunotherapy becomes more prevalent.

Biological Activity and In Vivo Half-Life of Pro-Activin A

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The family of transforming growth factor-β (TGF-β) proteins are master regulators of tissue homeostasis. Consequently, their deregulated activities are associated with a multitude of human disorders including, infertility, cancer, obesity, tissue degeneration, and fibrosis. Correcting TGF-β activity is an attractive approach to restore tissue homeostasis, but is limited by the poor in vivo stability of TGF-β proteins. The active proteins are derived from large Pro-TGF-β forms that undergo proteolytic maturation, yielding a pro:mature non-covalent complex, with pro and mature (active) domains. Prodomains are removed during commercial preparation, leaving only mature active ligand. These preparations, having half-lives of minutes, are unsuitable for therapeutic treatment in humans. The pro:mature non-covalent complex, in which the mature active ligand is shielded by its prodomains, is predicted have greater in vivo stability than the mature ligands. In this study, we examined whether the prodomain could reduce the clearance rate and increase activity in vivo for a well characterised member of the TGF-β family, activin A. To address this, we aimed to generate a pro:mature complex. To favour production of the Pro-activin complex, the native cleavage site was enhanced by site-directed mutagenesis. This modification improved the processing of activin precursor. Pro-activin complexes were isolated from stable HEK-293E cell lines by immunoaffinity using an antibody targeted to the prodomain. Importantly, the purified Pro-activin complex had comparable in vitro bioactivity to the commercially available mature preparations, supporting that the prodomain does not perturb activin bioactivity. In vivo work determined that the half-life of activin was improved two-fold, compared to the mature alone, and biological activity was also improved. Ongoing studies aim to further improve the half-life of activin A. The outcomes of this work will provide a blueprint for generating long-acting TGF-β ligands, which would benefit the treatment of human conditions associated with altered TGF-β signalling.

Pharmacokinetics of Leptin in the Gut of Mice

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Leptin is a protein hormone originally identified from adipose tissue and known for its effects on appetite. Leptin is now known to be produced in many tissues including the stomach, and our earlier work showed that when a physiologic dose was injected intravenously approximately 13 % of the dose was recovered intact from the lumen of the gastrointestinal tract (GIT) after 60 minutes. To examine the pharmacokinetics of leptin in the GIT, non-fasted mice were lightly anaesthetised before oral gavage of 12 ng of 125I-labelled leptin. Samples were analysed by gel permeation HPLC to confirm that the leptin was not degraded and the amount present was determined using a γ-counter. Radiolabelled leptin in the stomach declined from 53 % to 24 % of the administered dose 30 – 120 min post-gavage. A small peak (~ 4 – 8 % of the dose) appeared to move aborally through the small intestine, with approximately 4 % of the dose reaching the hindgut within the 2 h study. Throughout the experiment radiolabelled leptin was detected in the blood, with approximately 3.5 % of the dose calculated to be in the circulation at all times examined. The radiolabelled leptin in plasma was found to be 74 ± 6 % intact.

Here we show that leptin in the digestive tract moves aborally along the digestive tract, suggesting a role in the intestine. The gradual decline of leptin from the lumen of the stomach may indicate that leptin associates with digesta. We also report that leptin in the lumen of the gut was recovered intact from the blood. Our previous work has shown that leptin in the circulation is also recoverable from the lumen of the digestive tract, suggesting that leptin may be cycling between the gut and the circulation.

Is oxytocin receptor SNP rs53576 a potential biomarker for psychological resilience?

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There is an increasing focus on the positive psychological traits (optimism and resilience) rather than the negative psychological traits such as depression and anxiety in attempts to improve mental health. Negative psychological traits are associated with a number of biomarkers such as cortisol, alpha-amyase, 5-HTTLPR in association with stress. Recent studies show psychological resilience is heritable and it acts as a buffer between depression and stress. Several research groups are working towards a better understanding of resilience and in identifying reliable biomarkers of resilience such as telomere length, oxytocin (OXT) and SNPs of oxytocin receptor, reelin and other depression associated genes. OXT a neuropeptide
secreted in the hypothalamus is involved in a number of physiological and social behaviours and has a role in the development of social behaviours such as trust, positive communication, group favouritism, and reduced social stress. We hypothesized that the oxytocin receptor (OXTR) SNP rs53576 that results in Guanine (G) to Adenine (A) substitution may be associated with resilience as it has been shown to be associated with positive traits.

We collected DNA samples from buccal cells from a self-selecting community population and collected questionnaire data for depression and anxiety (Zung) and resilience scores (Connor Davidson). OXTR SNP rs53576 was analysed from 121 non-medicated subjects using traditional restriction enzyme digest, sequencing and qPCR-HRM methods. Results showed our cohort did not fit with HW equilibrium for OXTR SNP rs53576 (p = 0.00004) and did not show any association between OXTR rs53576 and to depression or resilience (p > 0.5). However further study with a larger cohort and including data from other OXTR SNPs may be worthwhile.

FGF9 activity from normal males and a 46,XY female

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Disorders of sex development (DSDs) include 46,XY gonadal dysgenesis (GD), where a specific molecular diagnosis is made in only ~30% of patients. Improved understanding of the genetic causes of DSD will lead to better diagnosis and management. FGF9 is expressed in Sertoli cells and is critical for testis determination in the mouse since Fgf9−/− mice show XY gonadal sex reversal. In the developing XY gonad FGF9 maintains Sox9 expression through repression of Wnt4. However, the mechanism of Wnt4 repression by FGF9 is still unknown. We have established an in vitro assay system of FGF9 function during foetal gonadal development to identify the signalling pathways involved in Wnt4 repression. We show that FGF9 treatment of the mouse Sertoli cell line 15P-1 can efficiently down-regulate Wnt4 expression in a dose dependent manner. Cycloheximide treatment inhibited Wnt4 repression, suggesting that FGF9 requires new protein synthesis to down-regulate Wnt4. FGF signalling activates four major signalling pathways; MAP Kinase, AKT, STAT, and the PLCγ. To determine which pathways are involved in FGF9 repression of Wnt4, we treated 15P-1 cells with drugs to these pathways. Drugs blocking the ERK1/2 and JNK pathways significantly inhibited Wnt4 repression, suggesting that FGF9 down-regulates Wnt4 via the ERK1/2 and JNK MAPK pathways, but not via p38 MAPK pathway. Testing in gonad cultures ex vivo is underway.

FGF9 mutation has not been described in human DSD. Here, we identified an FGF9 variant in a 46,XY GD patient, a maternally-derived heterozygous single nucleotide substitution, c.583G>A (p.Asp195Asn) using 1000 DSD gene targeted Massively Parallel Sequencing. Recombinant wildtype and the variant FGF9 protein have been purified and the variant protein inhibited FGF9 repression assay and ex vivo experiments are underway.

Castration Effects on the Expression of Kisspeptin and Rf-Amide Related Peptide-3 and their Co-Expression with Oestrogen Receptor a in the Ram Hypothalamus.

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The mechanism by which testicular hormones exert a negative feedback action is unclear, as GnRH neurons do not contain receptors for androgen or oestrogen. The RF-amides, Kisspeptin and RF-amide related peptide-3 (RFRP-3) could be potential neuronal pathways. In ewes, 93% of arcuate kisspeptin cells co-expressed ERα (1), with hypothalamic RFRP-3 cells expressing ERα ranging between 20% in mice (2) and 40% in Syrian hamsters (3). This study aimed to determine if castration influenced the expression of ERα in kisspeptin and RFRP-3 neurons in the ram. Dual label fluorescence immunohistochemistry for the co-expression of the RF-amides with ERα was used to compare the percentage of RF-amide cells containing ERα in the hypothalamus of intact merino rams and long term wethers (n=4/group), and in rams castrated 4 weeks previously or sham castrated rams, with ewe tissue (luteal phase) included for comparison (n=4/group). Ninety percent of kisspeptin cells expressed ERα in the caudal arcuate nucleus in wethers (long and short term) and ewes. Rams, by comparison, expressed very few kisspeptin cells, and these did not express ERα. Less than 1% of RFRP-3 neurons co-expressed ERα in the merino sheep regardless of group. By contrast, RFRP-3 fibres were in great abundance in intact rams. This suggests that kisspeptin expression and its co-expression with ERα is influenced by testicular hormones. The lack of co-expression of RFRP-3 and ERα in the ram suggests that oestrogen negative feedback in these animals is unlikely to involve RFRP-3 neurons.

F2 fetal nephron number and weight benefits of endurance exercise training for females born small on high fat diet

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Uteroplacental insufficiency is the major cause of intrauterine growth restriction in Western society and is associated with cardiorenal disease which is exacerbated by "second hits" such as pregnancy and overweight/obesity. We reported that F2 fetuses have nephron deficits which contribute to the development of F2 high blood pressure. This study determined if F2 male nephron deficits of mothers born small are exacerbated by a maternal high fat diet (HFD) and whether endurance exercise training can prevent these deficits.

Uteroplacental insufficiency was induced by bilateral uterine artery ligation (Restricted) or sham (Control) surgery on E18 in Wistar-Kyoto rats. Female offspring were fed a chow or high fat (43% kcals from fat) diet from 5 weeks to mating (20 weeks) and throughout pregnancy. Female rats were exercised on a treadmill 4 weeks before mating and throughout pregnancy. Male fetal nephron number was quantified using unbiased stereology and fetal and placental weights were measured at E20.

Restricted and Control female rats that were exposed to a HFD were heavier with more dorsal fat than females on a chow diet. Exercise prevented dorsal fat gain in Restricted HFD compared to sedentary. F2 male nephron deficit was present in mothers born small regardless of diet (-18-45%). A HFD reduced F2 male nephron number in Control mothers (-32%). Exercise prevented the HFD induced nephron deficits in F2 males of both Control and Restricted mothers. Despite no treatment effect on placental weight, exercise prevented the reduced fetal weight in females born small.

We demonstrated that females born small are at a greater risk of increased adiposity. F2 male fetal nephron deficits in mothers exposed to a HFD were prevented by the lifestyle intervention of endurance exercise. This may prevent the development of F2 high blood pressure.

Pituitary Metastases

Veronica Wong, Zoran Apostoloski

Publish consent withheld

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Management of Diabetes in Lung Transplant Recipients

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Diabetes mellitus (DM) is common in lung transplant (LTx) recipients and is associated with increased mortality. We conducted an observational study of all patients receiving LTx between 1/8/2010-1/4/2013 inclusive to determine current management of DM and insulin requirements over time. DM status was determined by oral glucose tolerance test performed pre-, 3 months, then annually after LTx. DM management was determined from medical records.
Of 174 patients in total, 37 (21%) had DM before and after LTx, and 40 (23%) developed DM post-transplant, which persisted throughout follow-up. A further 18 (10%) had transient DM, which subsequently resolved. Of those with diabetes both pre- and post-LTx, 19 (51%) used insulin pre-transplant. By 3 months, 33 (92%) required insulin and 24 of the surviving 28 (86%) remained on insulin at 2 years. In patients taking insulin pre-LTx, there was no significant change in mean insulin dose from pre- to 3 months post-LTx (34 (SD 21)–44 (19) units, p>0.05), even when adjusted for weight. There was also no difference in insulin dose between 3 months and 2 years, despite a significant fall in prednisolone dose over this time.

Most patients with new onset DM (32/40, 80%) were diagnosed by 3 months and 27/32 (84%) were on insulin at this time. Overall, 31/40 (78%) patients with new-onset diabetes required insulin. Two patients were managed solely with oral hypoglycaemic agents. Seven patients (18%) had dietary management.

Of the 18 patients with transient DM, 6 were treated with insulin. The remainder were diet controlled. Insulin was commenced by 3 months in all 6 patients at a mean dose of 15 units (0.22 units/kg) per day. Insulin is the mainstay of DM management following LTx. There was no significant change in insulin dose before and after LTx despite changes in prednisolone dose and clinical status.

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**Time-specific basal cortisol cut-offs are a more reliable predictor of passing a Synacthen Stimulation Test than a single threshold level.**

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Background: Cortisol is a glucocorticoid hormone with well-recognised patterns of secretion, including an ultradian rhythm which underpins a diurnal circadian rhythm of higher morning cortisol (morning acrophase) with night time nadir. Morning cortisol collection is important for assessment of adrenal sufficiency and levels from 300-500 nmol/L have been demonstrated in various studies to predict passing the Synacthen stimulation test (SST) with variable specificity ranging from 62-100%. Aim: Given the significant diurnal decline in cortisol across the morning, the aim of our study was to determine whether time specific reference intervals (multiples of the median – MoMs) for cortisol would have utility in predicting SST outcome, reducing the number of unnecessary tests. Methods: We calculated individual MoMs for discrete time intervals across the morning between 7:00am and 12 midday and performed ROC curve analysis to determine 90% and 95% specificity cut-offs within each time interval. Results: A single 95% specificity threshold applied across the morning showed variable specificity for predicting SST outcome (range: 91-100%). Using a MoMs approach for each discrete time interval yields a more consistent specificity across the morning (range: 95-100% at 95% specificity). Individual MoMs for discrete time intervals optimised specificity without compromising sensitivity (range: MoMs 75-89% versus single cut-off 58-84% sensitivity). Conclusion: Compared to a single cut-off value for basal morning cortisol, time-specific MoMs gives a more reliable prediction of passing a SST.

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**Tamoxifen reduces hepatic VLDL production in women: a possible GH-mediated mechanism for the development of fatty liver**

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Steatosis is a common complication of growth hormone (GH) deficiency. GH plays a vital role in lipid metabolism, stimulating hepatic fat oxidation and the synthesis of very low-density lipoproteins (VLDL) for export of triglycerides (TGs). We previously reported that tamoxifen suppresses the secretion and hepatic action of GH¹. We hypothesize that the GH-deficient state induced by tamoxifen, lowers the secretion of VLDL.

Objective: To investigate whether tamoxifen inhibits hepatic VLDL secretion.

Design: Eight healthy, normolipemic women (BMI 23.7±1.2 kg/m², age 64.4±2.2 years) were studied at baseline and after 2 weeks of tamoxifen (20 mg/d) treatment. We quantified apolipoprotein B (apoB), the structural protein of VLDL particles, by stable isotope 2H3-leucine turnover technique using steady state methodology. The enrichment of labelled leucine into VLDL-apoB was measured using gas chromatography mass spectroscopy. VLDL-apoB fractional catabolic rate (FCR) was determined using a multicompartment model. VLDL-apoB secretion was estimated as the product of FCR and VLDL-apoB concentration. Circulating levels of IGF-I, FFA, and TG were measured at baseline and following tamoxifen treatment.

Results: At baseline, mean VLDL-apoB concentration was 94±19.8 mg/L. VLDL-apoB FCR and secretion were 3.7±0.6 pools/d and 4.6±1.1 mg/kg/d, respectively. Tamoxifen significantly (p<0.05) lowered VLDL-apoB concentration and secretion by 27.6±7.8% and 30.7±9.8%, respectively. Tamoxifen also significantly lowered circulating IGF-I concentration (14.8±5.3%; p<0.05). There were no significant changes in plasma TG and FFA levels following tamoxifen treatment.
Summary: Tamoxifen significantly lowered VLDL-apoB concentration as a consequence of a lower production rate. Tamoxifen significantly reduced IGF-I, a hepatic marker of GH action.

Conclusion: The suppression of GH-IGF-I axis by tamoxifen is associated with lower rates of VLDL-apoB secretion. Diminished hepatic VLDL secretion may contribute to the development of fatty liver during tamoxifen therapy. Supported by the Princess Alexandra Hospital Research Support Scheme and the NHMRC Australia.

1. Birzniece et al., Paracrine regulation of growth hormone secretion by estrogen in women. JCEM 2010;95:3771-6

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Hypophosphataemic osteomalacia associated with iron infusions: Report of three cases
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Although the incidence of serious adverse reactions remains low with administration of parenteral iron, hypophosphataemia is increasingly being recognised as an important complication, though it is often transient and asymptomatic. A postulated mechanism for hypophosphataemia is the reduced degradation of FGF-23, resulting in renal phosphate wasting and reduced synthesis of 1,25-dihydroxy vitamin D.

We report two post-menopausal women who developed symptomatic hypophosphataemic osteomalacia with bone pain and multiple insufficiency fractures on a background of chronic gastrointestinal blood loss, necessitating monthly iron polymaltose infusions over 13- and 17-months, respectively. Respective blood tests revealed serum phosphate of 0.29 and 0.43 mmol/L [0.8 - 1.5 mmol/L], 25-hydroxy vitamin D of 98 and 57 nmol/L, 1,25-dihydroxy vitamin D of 80 and 32 pmol/L [60 - 158], alkaline phosphatase of 302 and 125 U/L [30 - 130], serum calcium and PTH. Urinary fractional phosphate excretion of the first patient was 24% [5%] with TmP/GFR of 0.47 [0.87 - 1.4], consistent with renal phosphate wasting. Serum FGF-23 obtained from the second patient was 285 pg/mL [≤54]. There was no biochemical evidence of Fanconi’s syndrome. Bone mineral density scans were in the osteoporotic range and whole body bone scans revealed increased uptake at multiple skeletal sites indicative of insufficiency fractures and in a pattern consistent with osteomalacia. Cessation of iron infusions resulted in clinical and biochemical improvement within 2-months.

The third case was a 25-year-old male with Crohn’s disease and iron deficiency anaemia who presented with severe hypophosphataemia (0.13 mmol/L) and generalised muscle weakness twelve days after a single dose of iron polymaltose. There was no arrhythmia on ECG. Serum calcium, PTH, 25-hydroxy and 1,25-dihydroxy vitamin D were normal with supplementation. Fractional phosphate excretion was marginally elevated (6.5%), reflecting depleted phosphate stores. Bone mineral density scan was in the osteoporotic range. Following oral phosphate supplementation, serum phosphate and metabolic bone parameters normalised within 2-months. Vigilant prescribing of parenteral iron is needed to avoid clinically serious hypophosphataemia.

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Extremes of autoimmune thyroid dysfunction associated with interferon treatment in one patient
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Autoimmune thyroid disease associated with interferon therapy occurs in 2.7 to 10% of patients and at a median time of 17-weeks (range 4 weeks–23 months) after beginning interferon therapy. Destructive thyroiditis, Graves’ Hyperthyroidism and autoimmune (often subclinical) hypothyroidism have been described, the latter occurring in 87% of cases and persisting in 2/3 of cases whereas destructive thyroiditis occurs in 1/3. Thyroid replacement or anti-thyroid therapy are indicated in autoimmune hypo- and hyperthyroidism, respectively, with continuation of interferon. However, in destructive thyroiditis, cessation of interferon may be temporarily necessary. Little is known about the development of the extremes of autoimmune thyroid disease activated by the undesirable immunomodulatory effects of interferon treatment, especially within a single patient, as reported below.

A 60-year old man with no prior history of thyroid disease received 48-week pegylated interferon and ribavirin therapy for chronic HCV with achievement of sustained virological response. Six months into treatment, he reported fatigue, weight gain and slowed cognition. Examination was normal. Serum TSH was 58.8 mIU/L [0.27 – 4.2], fT4 11.1 pmol/L [12 – 25], and fT3 4.2 pmol/L [2.5 – 6.0] with elevated anti-TPO (983 IU/mL, <35) and anti-TG (733 U/mL, <80) antibodies. He was commenced on thyroxine 100mcg daily with initial clinical and biochemical resolution but developed symptoms of hyperthyroidism with weight loss and tremor 14-months later. Serum TSH was <0.02 mIU/L, fT4 54.3 pmol/L, fT3 20.2 pmol/L, with an elevated TRAb of 4.0 U/L (<1.0), anti-TPO (1,163 IU/mL) and anti-TG (114 U/mL) antibodies. Technetium scan confirmed Graves’ Disease with bilateral diffuse increased tracer uptake (5.9%, 0.5 – 3.5%). The patient was commenced on carbimazole 15mg daily for 6-months. He self-ceased therapy with serendipitous clinical and biochemical remission (TSH 3.84 mIU/L, fT4 17 pmol/L, fT3 4.5 pmol/L, anti-TPO 383 IU/mL, anti-TG 23 U/mL, TRAb <1U/L).
Increased fat mass contributes to increased insulin resistance in men undergoing androgen deprivation therapy for prostate cancer.

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Background and aims: While androgen deprivation therapy (ADT) has been associated with insulin resistance and increased diabetes risk, there have been few controlled prospective studies. We hypothesized that ADT influences insulin resistance indirectly, via effects on body composition.

Methods: This prospective case-control study recruited 63 men with localised prostate cancer, 29 cases (newly commencing ADT) and 24 controls (not receiving ADT), matched for age and radiotherapy. Fat mass, lean mass and visceral adipose tissue (VAT) was measured by DEXA and insulin resistance was estimated from the updated Homeostasis Model Assessment (HOMA2-IR). Using a mixed model, the mean adjusted differences (MAD) between groups from 0 to 12 months are reported.

Results: Compared with controls, fat mass increased in men receiving ADT by 3529.5g [2012, 5047], p<0.02 and lean mass decreased by 1491g [181, 2801], p<0.02. VAT was unchanged (p=0.66). HOMA2-IR increased in the ADT group compared with controls (mean adjusted difference 0.59 [0.24, 0.94], p<0.02). HbA1c levels and prevalence of diabetes was unchanged. Increase in HOMA2-IR was predicted by a change in testosterone (p<0.001) or change in fat mass (p<0.001) in separate models, which...
were also strongly associated with each other (p>0.001). HOMA2-IR was not predicted by lean mass. In a combined model with testosterone and fat mass only, fat mass change (p<0.001) remained a significant predictor of HOMA2-IR, but not change in testosterone (p=0.63).

Conclusion:
These findings suggest that ADT may increase insulin resistance indirectly, via body composition changes, rather than via effects of testosterone withdrawal. This occurs in the absence of obvious changes in VAT, suggesting that there may be deleterious effects of subcutaneous fat. This reinforces the importance of implementing lifestyle measures to prevent obesity in men commencing ADT.

Ovarian Reserve of Women with Germline BRCA1 or BRCA2 Mutations.
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Background: Anti-müllerian hormone (AMH) is a surrogate marker of fertility; higher levels are associated with greater ovulatory potential. This study examined AMH levels of BRCA1 and BRCA2 mutation carriers and their non-carrier blood relatives.

Methods: Eligible women were from families segregating BRCA1 or BRCA2 mutations, enrolled in the Kathleen Cuningham Foundation Consortium for Research into Familial Breast Cancer (kConFab). Each woman had been tested for the family mutation, had completed an epidemiological questionnaire and provided a blood sample at cohort entry. Women were aged 25-45 years, with no personal history of invasive cancer, had not undergone oophorectomy and were not pregnant or breastfeeding at the time of blood draw. AMH was tested on stored plasma samples using an electrochemiluminescence immunoassay platform. Associations between AMH level and carrier status were tested by linear regression, using the natural logarithm of AMH as the outcome variable, carrier status as the explanatory variable, and adjusting for age at blood draw, oral contraceptive use, BMI and cigarette smoking.

Results: AMH level was measured for 693 women, 172 carriers and 216 non-carriers from families carrying BRCA1 mutations, and 147 carriers and 158 non-carriers from families carrying BRCA2 mutations. Within both groups, mutation carriers were younger at blood draw than non-carriers (p ≤ 0.031). BRCA1 mutation carriers had, on average, 25% lower AMH levels than non-carriers (p = 0.022). There was no evidence of an association for BRCA2 mutation carriers (p = 0.94).

Conclusions: This study suggests that women with a germline mutation in BRCA1 may have reduced ovarian reserve. This could have implications for their fertility, family planning and age at menopause.

Histological skeletal muscle changes in men with prostate cancer undergoing androgen deprivation therapy.
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Background: Androgen deprivation therapy (ADT) is an effective treatment for prostate cancer but has many adverse effects consequent to severe hypogonadism. Muscle mass declines with ADT, however changes at a histological level have not been studied in humans. In testosterone replacement, an increase in cross-sectional area of all fibre types is seen; therefore we hypothesised that in men undergoing ADT the opposite would occur.

Aim
To assess histological changes in skeletal muscle in men initiating ADT for prostate cancer.

Methods
This prospective cohort study involved obtaining percutaneous thigh muscle biopsies (vastus lateralis) from 9 men with localised prostate cancer. The samples were taken immediately before and 1 month (mean 30.3±4.1 days) after commencing ADT and immediately processed. Direct histology was performed to measure fibre size (H&E stains), fibre type distribution (ATPase and NADH stains) and mitochondrial activity (COX/SDH stains). Slides were also reviewed for lipid and glycogen content.
Results
Mean total testosterone decreased from 16.5 nmol/L at baseline to 0.4 nmol/L 1 month post-ADT (p=0.008). There was no significant change in mean fibre size (pre-ADT 61.5±19.1µm, post-ADT 56.8±7.8µm, p=0.95) or fibre type distribution (ratio pre-ADT 1.36, ratio post-ADT 1.15, p=0.43) over time. The variability coefficient for fibre size increased post-ADT (p=0.04), indicating an increased range of fibre sizes in post-ADT muscle compared to pre-ADT. No mitochondrial abnormalities or changes in intramuscular fat or glycogen content were noted.

Conclusions
No consistent histological changes were identified 1 month post-ADT. Increased fibre size variability post-ADT may be a consequence of testosterone fluctuations related to a transient rise in testosterone levels after ADT followed by the rapid fall to castrate levels. Further longitudinal studies are required to assess changes in muscle morphology and their functional consequences following prolonged exposure to hypogonadism.

Quality of life decrements in men with prostate cancer undergoing androgen deprivation therapy.
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Background
Androgen deprivation therapy (ADT), an effective treatment for prostate cancer has adverse effects consequent to severe hypogonadism. Effects on quality of life (QoL) are poorly characterised, due to limited evidence from controlled prospective studies. We hypothesised that men undergoing ADT will have decreased QoL in all domains.

Aim
To assess changes in QoL and to investigate contributing factors in men undergoing ADT.

Methods
Sixty-three men with prostate cancer were evaluated in a prospective, 12 month case-control study including 34 cases newly commencing ADT and 29 prostate cancer controls not receiving ADT, matched for age and radiotherapy. Participants performed the Short Form-12 (SF-12) (physical and mental components, and Aging Males’ Symptoms Score (AMSS) (somatic, sexual and psychological components) QoL questionnaires at 0, 6 and 12 months. Using a mixed model, the mean adjusted differences (MAD) in QoL scores between groups from 0 to 12 months are reported.

Results
QoL as measured by SF-12 showed decrements in the physical component for the ADT group compared with controls (MAD 3.56 [0.45, 6.68] p=0.026) but there was no significant difference in the mental component (MAD 1.22 [-2.23, 4.67], p=0.49). QoL as measured by total AMSS was worse in the ADT group compared with controls (MAD -9.48 [-13.04, -5.91] p<0.001). Deficits were seen in the somatic (p<0.001), sexual (p<0.001) and psychological components (p<0.044). The decrease in QoL by AMSS was related to increase in hot flushes (p=0.002) but unrelated to haemoglobin levels (p=0.45).

Conclusions
Men receiving ADT have decrements in somatic and sexual aspects of QoL exceeding the impact of the cancer diagnosis and radiotherapy alone. Changes in psychological well-being are less consistent, perhaps due to insensitivity of questionnaires to detect small changes. The observed deficits should be useful in patient counselling and implementation of targeted strategies to mitigate adverse effects of ADT.

Comparison of the insulin tolerance test against the glucagon stimulation and short Synacthen tests in patients with suspected hypopituitarism.
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Background: The insulin tolerance test (ITT), glucagon stimulation test (GST) and short Synacthen tests (SST) are employed in the evaluation of suspected cortisol and/or GH deficiency, with ITT considered the gold standard. We hypothesised that these dynamic tests may yield discordant results within individuals.

Methods: We performed a retrospective audit of adults who had undergone ITT plus either GST and/or 250mcg SST. Cortisol adequacy was locally defined as peak cortisol >550nmol/L at any time on ITT or GST, and at 30min on SST. GH adequacy was locally defined as peak GH >10mU/L at any time on ITT or GST. The primary outcome was discordance in cortisol and/or GH responses between the dynamic tests.

Results: Of 14 patients, 8 had ITT+GST and 7 had ITT+SST (including 1 patient who had all tests). Mean peak cortisols from ITT and GST in subjects who underwent both tests were 423 and 428nmol/L, respectively. In subjects who underwent ITT and SST, mean peak cortisols were 409 and 491nmol/L, respectively. Mean peak GH from ITT and GST in subjects with both
results were 4.3 and 16.6mU/L, respectively. In total, 9 of the 14 patients had discordant results using the defined decision points. Of the 5 patients with cortisol discordance, 3 were cortisol-adequate on ITT and inadequate on GST or SST, whilst 2 were adequate on GST and inadequate on ITT. The 5 patients with GH discordance were all GH-adequate on GST and inadequate on ITT.

**Conclusions:** Cortisol and/or GH discordance was found in 64% of patients. Glucagon and Synacthen appeared more potent stimuli of hormone secretion than hypoglycaemia, consistent with recent data. However, 3 subjects showed cortisol adequacy on ITT and not on GST or SST suggesting inter- or intra-individual variability. We recommend centre-specific and test-specific decision points be considered in dynamic tests of suspected hypopituitarism.

2. Simsek Y et al., Clin Endocrinol 2015; 82:45.

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**Fine needle aspiration of the thyroid: correlation with final histopathology in a series of 187 patients.**

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**Background:**

The risk of malignancy associated with thyroid nodules is ~5-15%. The Bethesda classification stratifies the risk based on fine needle aspiration (FNA) cytology and is used to guide management. However, false negatives remain a concern and is estimated between 1.3-11.5%. This study examined the accuracy of thyroid FNA by comparing the results with final histopathology, and evaluating the sensitivity, specificity and predictive values of FNA for the diagnosis of thyroid malignancy.

**Methods:**

Medical records of 449 patients who underwent FNA for thyroid nodules whom 187 were operated and have final pathological diagnosis were retrospectively reviewed. FNAs were classified according to the Bethesda classification. We calculated the malignancy risk for each category by follow up histopathology in all 187 cases that underwent subsequent surgeries at our institution.

**Results:**

Of the 550 FNAs performed, 187 cases proceeded to surgery (thyroidectomies or hemithyroidectomies). Malignancy rates at our institution were 21.05% for the non-diagnostic group; 10.0% for benign group, 44.44% for follicular lesion of undetermined significance (FLUS) group, 43.75% for the suspicious for follicular neoplasm group, 71.43% for the suspicious for malignancy group and 94.74% for the malignant group.

Sensitivity was 83.33%, specificity 71.29%, PPV 57.97%, NPV 90.0%, and diagnostic accuracy was 75.17%.

**Conclusions:**

Thyroid FNA has high sensitivity and specificity, but false negative and false positive results cause concern. It is difficult to calculate the true frequency of false negatives because only a small percentage of patients with benign FNA undergo surgery. Our findings do not match the published data. Our malignancy rate is higher for the benign group (10%) compared to published literature of 0-3% with a benign FNA result. This suggest that when making treatment recommendations and counselling patients, we should use data from our own institution in addition to published values.


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**Transient Hypercalcaemia in Hospitalised Elderly Patients: an Association with Underlying Hyperparathyroidism and Vitamin D Supplementation**

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**Introduction**

Hypercalcaemia is commonly seen in hospitalised patients, with a common aetiology being primary hyperparathyroidism. It has been observed that many elderly patients admitted with an acute illness have transient hypercalcaemia. It is unclear whether this group of patients has mild underlying hyperparathyroidism.

**Objective**

To determine 1) the incidence of primary hyperparathyroidism in patients with transient hypercalcaemia 2) the contribution of calcium and vitamin D supplements in the development of transient hypercalcaemia

**Methods**
A retrospective analysis of laboratory data and medical records of patients with hypercalcaemia (defined as corrected serum Ca of >2.60) and normocalcaemia, was performed. Vitamin D levels, renal function, parathyroid hormone (PTH) and medications were also analysed.

**Results**

A total of 982 medical inpatients had their serum calcium checked between June-Dec 2013. A total of 104 (10.6%) patients (F 65/M 39, mean age 79 years) had transient hypercalcaemia, with normalisation of calcium during or after admission. A small proportion, N=25/104 (24%) had PTH checked; 10 of those 25 (40%) had elevated PTH and 15 (60%) had an inappropriately normal PTH. None had a suppressed PTH.

101 normocalcaemic patients (F 51/M 50, mean age 75 years) were also analysed as a control group. The proportion of patients with acute kidney injury (AKI) was similar in both groups (P = 0.382).

Calcium supplement intake was similar between the two groups (P=0.233), however there was a significantly higher rate of vitamin D use in the transient hypercalcaemic group (P=0.020). Interestingly, thiazide use was higher in the normocalcaemic group (P = 0.008).

**Conclusion**

Transient hypercalcaemia is common in hospitalised elderly patients. Hyperparathyroidism was the likely cause in all patients who had PTH measured. It was found that vitamin D supplementation appeared to be associated with transient hypercalcaemia, however calcium supplementation and AKI did not.


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**Timely Commencement of Anti-resorptive Therapy Post Fragility Fractures: A Discrepancy Between Recommendations and Clinical Practice**

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**Introduction**

Current evidence suggests that early rather than late administration of bisphosphonates prevents re-fracture after fragility fractures. [1] It has been previously proven that there remains a significant treatment gap in the prescription and timing of anti-fracture therapy. [2]

**Objective**

To determine 1) whether patients with fragility fractures are receiving anti-resorptive therapy and the time frame in which this occurs 2) the recognition and treatment of vitamin D deficiency in these patients.

**Methods**

A retrospective analysis of medical records and laboratory data of patients with fractures was performed. Vitamin D levels, renal function and management of fractures were also analyzed.

**Results**

A total of 205 patients (F 154/M 51, mean age 80 years) presented to Box Hill Hospital with fractures from June-Dec 2013. The most common fracture was femur (N=112, 60%), followed by humerus (N=44, 21%) and Colles (N=36, 18%). Out of 180 patients with osteoporosis, only 32 (17%) had bisphosphonates started, at a mean time of 26 days. Forty-seven (27%) patients were commenced on vitamin D, whilst 7 (4%) patients were started on calcium. Seventy (41%) out of 107 patients had vitamin D deficiency, however less than half (N=33, 43%) were treated. Initiation of anti-resorptive therapy was predicted in patients with a history of osteoporosis (P = 0.002), Caucasian ethnicity (P = 0.049) and femoral fractures (P=0.029). Others including age (P = 0.323), gender (P = 0.408) and osteoporotic risk factors (P = 0.108) did not influence the decision to start therapy.

**Conclusion**

Fragility fractures and vitamin D deficiency do not appear to be treated with adequate pharmacological therapy. Measures need to be undertaken to improve awareness amongst medical practitioners.

"Parachutes to Prevention" – A conceptual change in acute adrenal insufficiency education

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Prevention of adrenal crisis has been the focus of care for individuals with primary and secondary adrenal insufficiency. The key to prevention is through patient and health professional education. Recognition of impending adrenal crisis is often missed as patients may appear clinically stable initially and health professionals are not aware that they can deteriorate rapidly. We developed a “parachute” concept called “Parachutes to Prevention” as a tool to better illustrate in pictorial form the elements considered critical in the prevention and treatment of acute adrenal insufficiency. This was presented at the Sydney Chapter of the Australian Addison’s Disease Association (AADA) annual meeting recently and a survey of the efficacy of the tool pre- and post- presentation was conducted.

Twenty-five participants completed a questionnaire. Twenty one (84%) were female with a mean age of 48.3yrs and average duration of adrenal insufficiency (since diagnosis) of 5.6yrs. All participants spoke English at home. This was the first Addison’s Awareness meeting for 40% of the respondents.

Participants were asked several questions around their management of sick days. They were then given a 20-minute presentation using the ‘Parachutes to Prevention’ tool. Following this, a repeat questionnaire demonstrated a significant ease in the number of safety measures that individuals could nominate for themselves, with a median increase of 5 additional preventative measures. Furthermore, they were able to individualise their own set of parachutes.

This tool was also used recently at Emergency Department nurses’ education sessions and resulted in strongly positive feedback from paramedical staff who indicated that these simple, yet clear, images were imprinted in their memory.

Given the success of the initial education sessions with this tool, we are now working with the Sydney AADA group to further develop the “Parachutes to Prevention” concept. This includes its application within the high risk non-english speaking group.

A case of primary amenorrhoea and hyperandrogenism

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We report the case of a 42 year old female with Müllerian agenesis and hyperandrogenism, with a possible unifying diagnosis of a WNT4 gene mutation. The patient presented with primary amenorrhoea aged 15. She had characteristic features of Müllerian agenesis: normal secondary sex characteristics, female external genitalia, a vaginal introitus but no true vagina, absent uterus on imaging and at laparoscopy, and a single right kidney. Karyotype was 46XX. There was no definite ovary located initially, though imaging later revealed a 22mm soft tissue mass in the region of the vaginal vault which remained stable in size over subsequent decades. This was presumed to be ovarian tissue as the patient had pre-menopausal range oestradiol and biochemical evidence of ovulation. She received no further medical care until age 28, when she was noted to have hirsutism and acne. There was mild biochemical hyperandrogenism but 17α-hydroxysteroid dehydrogenase and 17α-hydroxylase, which are required for the production of testosterone and are normally suppressed in the female, were present in the patient's circulating hormone profile. We are pursuing WNT4 genetic testing in this patient.


### An Odd Hot Spot

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We report the case of a 47 year old man with papillary thyroid cancer (PTC) presenting with a toxic thyroid nodule. The patient had lethargy, dysphonia and biochemical hyperthyroidism. Thyroid ultrasound showed a 43mm nodule in the right lobe, with coarse internal calcification and vascularity. The nodule was hot on technetium uptake scan. Fine needle aspiration (FNA) was recommended given the nodule’s size and presence of calcification. FNA cytology was consistent with PTC. He underwent total thyroidectomy and central neck dissection. Histopathology confirmed a moderately differentiated 50 x 40 x 30mm PTC replacing the right lobe with metastatic disease in 2 of 6 central compartment lymph nodes.

The 2009 American Thyroid Association (ATA) Guidelines do not recommend cytological evaluation for hyperfunctioning nodules, as they are believed to rarely harbour malignancy (1). However, Mirfakhraee et al. reviewed the prevalence of thyroid cancer within solitary hot nodules as reported by 14 surgical case series and found rates of intranodular carcinoma ranged from 0 to 12.5%, with a weighted total mean of 3.1% (2). In children, the risk of differentiated thyroid cancer in hot nodules may be as high as 29% (3).

However, no studies have specifically examined the validity of high-risk features (historical and ultrasound) or accuracy of cytology in the diagnosis of toxic thyroid cancers. Hot nodules were specifically excluded from some studies of sonographic predictors of malignancy (4) which formed the basis for the ATA’s recommendations (1). Moreover, increased intranodular vascularity occurs in 73% of all hyper-functioning nodules (5), so should not be considered a risk factor for malignancy in hot nodules. Thus, while the presence of differentiated thyroid cancer in toxic nodules may not be as rare as previously thought, detection remains challenging.


### Calcium stimulation test to localize insulinomas- Local centre experience

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Introduction: Non-invasive imaging modalities are often unable to localize insulinomas. Localization through calcium stimulation test is often dependent on expertise of the operator.

Aim: To assess the accuracy of calcium stimulation in diagnosis of cause of hypoglycemia at a tertiary referral centre.

Method: This is a retrospective analysis of a single centre experience in Newcastle, Australia from 2001 to 2015.

Results: 14 consecutive patients, 8 females and 6 males with mean age 33.5 years (range 25-42) were investigated for insulinoma over the past 14 years at John Hunter Hospital, Newcastle. Calcium stimulation test was performed on all patients by injecting calcium gluconate 0.025 mEq/kg directly into the arteries supplying the pancreas and liver. Samples were collected from the hepatic vein at -120,0, 30, 60,90, 120, 180 seconds. The results of the study were compared with the intraoperative and histological findings in 9 patients. The findings were also compared with other imaging modalities. Preliminary analysis showed that 2/14 had MEN 1 syndrome. 9/14 patients had insulinoma. 1/14 factitious disorder, 1/15 congenital hyperinsulinism. 2/14 had post gastrectomy hyperinsulinemia. Calcium stimulation test identified insulinoma correctly in all 9 cases. It was truly negative in 3 cases (factious, congenital hyperinsulinism, post gastrectomy hyperinsulinemia). It was falsely positive in 1 case of post gastrectomy hyperinsulinemia.

Of these 9 cases of insulinoma only 3 were identified on CT scan and 1 on MRI. Indium octreotide was done in 3 cases and was falsely negative in all 3. Gallium dotatate was done in 3 cases and was true positive in 1 case and truly negative in 2 cases.
Conclusions: Calcium stimulation test remains the investigation of choice for localizing insulinoma. Expertise at our centre was comparable to other centres in the world. Of all the other non-invasive imaging modalities, gallium dotatate scan was the best performing.


IGF-1/IGFBP-1 axis are closely associated with insulin secretion in Korean children.

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Background : The IGF system is involved in the development of metabolic and cardiovascular disease. This study aimed to investigate the association of insulin-like growth factor-1 (IGF-1), IGF-binding protein-1 (IGFBP-1) and IGFBP-3 with insulin resistance and type 2 diabetes in children.

Methods : We included 36 children aged 10 to 16 years without known diabetes, medication, chronic disease. They were classified into 3 groups according to the results of oral glucose tolerance test and other clinical/laboratory findings. We performed anthropometric measurement and laboratory tests. The fasting levels of serum IGF-1, IGFBP-1 and IGFBP-3 were measured.

Results : 1) Serum IGF-1, IGFBP-3 and IGF-1/IGFBP-1 molar ratio levels were significantly higher in glucose intolerance group. Serum IGF-1(r=0.396, P=0.023) and IGFBP-3(r=0.628, P<0.001) had negative correlation with IGFBP-1. 2) Serum IGFBP-1 was negatively correlated with age, body mass index (BMI), systolic blood pressure, serum c-peptide, insulin, and HOMA-IR. And serum IGF-1/IGFBP-1 was significantly related with serum c-peptide, insulin and HOMA-IR. 3) Serum IGFBP-1 had no correlation with fasting plasma glucose level, lipid profile, apoprotein A/B and HbA1c. It was not different between normal glucose tolerance group and glucose intolerance group. 4) In normal glucose tolerance group, serum IGF-1 and IGF-1/IGFBP-3 was no significantly different between obese and non-obese groups. But IGFBP-1 had negatively associated with age, BMI, systolic blood pressure, serum c-peptide, IGFBP-3 and HOMA-IR.

Conclusion: Serum IGF-1/IGFBP-1 molar ratio was significantly elevated in Korean children with glucose intolerance sate and especially, serum IGFBP-1 correlated with serum c-peptide. These findings suggest that IGF-1 may related glycemic control and insulin secretion in children.

Utility of FDG-PET CT scanning in succinate dehydrogenase B mutation related lesions

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Context: Mutations of the gene encoding Succinate Dehydrogenase B (SDHB) are associated with a highly penetrant phenotype that includes paragangliomas, phaeochromocytomas and renal cell carcinoma.1 Patients with mutations of SDHB require lifelong surveillance, however there is currently no consensus regarding optimal screening regimens.2,3 Due to abnormal glycolytic processing and delay in 18F-fluorodeoxyglucose (18F-FDG) clearance, 18F-fluorodeoxyglucose positron emission tomography with computed tomography (18F-FDG-PET/CT) imaging has theoretical advantages for imaging benign and malignant SDHB mutation-related neoplasms.4

Objective: Determine sensitivity and specificity of 18F-FDG-PET/CT compared to other modalities for SDHB mutation related lesions.

Design: A retrospective audit reviewed adult patients with confirmed SDHB mutation who underwent 18F-FDG-PET/CT at our institution between 1/7/2011 and 30/5/2015. Lesions numbers and locations detected by 18F-FDG-PET/CT were compared to those on CT and any other imaging modalities or histology available.

Results: 26 18F-FDG-PET/CTs were completed on 20 patients during an average follow up was 53 months (range 2-156). 18F-FDG-PET/CT compared to CT showed no additional lesions in 3 of 4 positive studies (75%) with a false positive uptake in the surgical bed of a carotid body tumour in 1 study, and 0 missed lesions in 4 of 4 positive 18F-FDG-PET/CTs. PET more accurately detected bony disease for metastatic paraganglioma than MIBG, but was similar to GaTate, MRI and CT. 22 18F-FDG-PET/CTs (85%) showed no abnormality; of 21 scans with other imaging for comparison, there were 0 missed lesions. 8 of 22 (36%) negative 18F-FDG-PET/CTs correlated with contemporary (within 6 months before) or later CT results, and 4/22 (18%) with other imaging. 9 of 22 (41%) negative 18F-FDG-PET/CTs correlated with other imaging done ≥6 months prior.
Conclusions: In patients with SDHB mutation, 18F-FDG-PET/CT was at least as sensitive and specific as other imaging modalities, both for metastatic and non-metastatic disease, and may detect bony metastatic disease better than MIBG.


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**Hypokalaemia Post-Saline Suppression Test in Primary Hyperaldosteronism**

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**Background:**

Primary hyperaldosteronism (PHA) accounts for 5-10% of patients with hypertension (1). Saline suppression test (SST) is a commonly used confirmatory test in the diagnosis of PHA. Although potassium (K) is checked at baseline with recommendations to adequately replace prior to SST, there are no recommendations to routinely check potassium post-SST. This contrasts guidelines for the fludrocortisone suppression test (FST) which is known to cause hypokalaemia. A previous study monitored K levels post-SST in a subgroup of patients, and found a non-significant decrease (-0.05 +/-0.2mmol/L) in potassium levels post-SST (2). We report a retrospective series of patients who became hypokalaemic in the 2 hour period post-SST.

**Methods:**

A retrospective audit was conducted of patients with confirmed PHA who underwent SST between 2005 and 2015. Pre- and 2 hour post-test potassium, aldosterone and renin levels were measured. Results are expressed as mean ± standard error of the mean (SEM) and number (%).

**Results:**

Twenty five patients were included in the final analysis; 13 (52%) were males, and mean age 53 ± 10.5 years. Overall, there was no difference in the mean pre- and post-SST potassium levels (p=0.08). However, there was an inverse correlation between pre-SST K and the change in post-test K levels (p=0.01); with the highest pre-test K patients experiencing the greatest decline in post-K levels. Eight (32%) were hypokalaemic (K<3.5mmol/L) pre-SST and required intravenous or oral K supplements.

For patients that were normokalaemic pre-SST, there was a significant decrease in serum potassium levels post-SST (3.7±0.05 vs. 3.5±0.08, p=0.01). Seven subjects (41%) who were normokalaemic pre-test became hypokalaemic post-SST; and 5 (29%) remained hypokalaemic on day 2.

**Conclusion:**

Hypokalaemia is common post-saline suppression test in primary hyperaldosteronism. The pathophysiology remains unclear. We recommend that potassium levels be routinely measured post-test and on day 2 to detect persistent hypokalaemia.


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**Dilemmas In The Diagnosis Of Cushing’s Syndrome In The Acutely Unwell Patient**

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The distinction between Cushing’s Syndrome (CS) and Pseudo-Cushing’s Syndrome (PCS) can be difficult: more difficult in acutely unwell patients. PCS occurs in patients with systemic conditions that activate the hypothalamic-pituitary-adrenal (HPA) axis; the principle mediator of a stress response. This case illustrates the difficulties in diagnosing CS during critical illness; and the effects of critical illness on the HPA axis.

A 36 year old male was admitted with subacute combined degeneration of the cord secondary to B12 deficiency, following progressive, debilitating limb weakness, paraesthesia and ataxia. His admission was complicated by intestinal pseudo-obstruction.
He appeared overtly hypercortisolemic, with moon facies, buffalo hump, supraclavicular fat pads, marked purplish-red striae (>1cm width) and had proximal myopathy (Fig. 1a & 1b). He denied any exogenous steroid use but history was significant for alcohol dependence, averaging 28 standard drinks (SD) daily.

Screening tests for CS revealed: elevated midnight salivary cortisol 32.9nmol/L (normal <10nmol/L), failure to suppress cortisol levels following an overnight low-dose 1mg dexamethasone suppression test (DST) (cortisol 210nmol/L), and detectable ACTH (21ng/L). However, a 24-hour urinary free cortisol was normal. The remainder of his hormone profile appeared to show deficiencies of gonadotrophins (LH 0.7 IU/L, FSH 0.4 IU/L, testosterone 1.7nmol/L) and the somatotroph axis (IGF-1 7nmol/L (15-40), GH 0.7ug/L). Thyroid hormone axis was intact.

Following near-recovery ten days later, repeat low-dose followed by high-dose DST now showed appropriate cortisol suppression. His gonadotroph and somatotroph axes also normalized. Post-hospital discharge, his alcohol intake has reduced significantly (3 SD/ week); with substantial loss of his previous phenotypic Cushingoid features (Fig. 2).

We report an uncommon cause of PCS secondary to longstanding alcoholism and critical illness. Rapid restoration of normal pituitary axis function was seen with resolution of illness and alcohol abstinence. We highlight some of the difficulties in the diagnosis of CS during critical illness.


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**Treatment Resistant Papillary Thyroid Cancer**

_Imaging of papillary thyroid cancer, showing calcifications and extrathyroid extension._

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_Synopsis_

Investigations for TSH elevation revealed a multinodular goitre. Progress ultrasound suggested malignancy and a follicular neoplasm was confirmed on biopsy. A total thyroidectomy was followed by radioactive iodine (RAI) ablation. Histopathology revealed papillary thyroid cancer (insular variant) with extensive capsular and vascular invasion.

Uptake in the T1 and L2 vertebrae was noted post therapy. Further RAI was administered but thyroglobulin remained elevated (1380 mcg/L). Resection of metastatic T1 and L2 lesions were undertaken. A 3rd dose of RAI was administered and thyroglobulin levels measured 1089 mcg/L pre-treatment. Thyroglobulin levels continued to rise (2252mcg/L prior to 4th RAI treatment), with new lung and recurrent bone metastases developing. Selected lesions were 18 FDG-PET positive. Due to a paucity treatment options, a 5th RAI dose was administered (total dose of 21.6GBq). External beam radiotherapy was delivered to the T1 lesion.

_Discussion_

RAI resistance occurs when there is (1) no uptake on post therapy scan, (2) progression of disease and (3) rising thyroglobulin. Although the metastases appeared iodine avid in this case, anatomical and biochemical response was lacking. These lesions corresponded to FDG-PET, reflecting a mixture of well and less well differentiated cell types.

The optimum I131 dose to treat metastatic disease remains unclear. Higher doses are used when increased risk is perceived based on clinical and histopathological features. This dose can range between 3.7-7.4Gbq but data addressing the optimal therapeutic and safe accumulative dose is lacking. Secondary malignancies have been associated with RAI therapy and are dose dependent. A meta-analysis has shown a 1.19 relative risk of secondary malignancies in patients receiving treatment for TC.

Tyrosine kinase inhibitors are currently being investigated and used as treatment for iodine refractory TC. However, access to these drugs in Australia is limited and if available, occurs in the setting of clinical trials.

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**Subclinical hypothyroidism in pregnancy related to TSH receptor blocking antibodies: An unique clinical conundrum**

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TSH receptor auto-antibodies (TrAb) belong to a heterogeneous group of auto-antibodies that may stimulate or inhibit TSH receptors. Most commonly, they exhibit an overall stimulatory effect and are associated with Grave’s disease. Rarely, they may exert a greater inhibitory effect, giving rise to hypothyroidism (1,2).

TSH binding inhibition immunoglobulin (TBII) assays are competitive immunoassays, which measure TrAb concentration. They do not inform about the biological effects of TrAb. Overall biological effects of TrAb are determined by their ability to stimulate cyclic AMP generation in thyroid stimulating immunoglobulin (TSI) bioassays. The behavior and proportion of these auto-antibodies may fluctuate with time and in response to treatment, changing the patient’s thyroid status.

Here, we describe a middle-aged Chinese lady with subfertility related to subclinical hypothyroidism due to blocking TrAb. She was treated with levothyroxine for 1 year before achieving TSH normalization and successful conception via in-vitro fertilization (Table 1).

Serial thyroid function monitoring during pregnancy revealed primary hyperthyroidism. Levothyroxine was stopped at 18 weeks of gestation with normalization of thyroid function (Table 2). At this time, the TrAb showed predominantly stimulating effects on TSI bioassay, which concurred with the switch in thyroid function.

Our patient mirrors previously described cases of hyperthyroidism resulting from a switch of TrAb from blocking to stimulating nature amongst middle-aged Japanese females (3–6). The proposed mechanisms include polarization of dendritic cells after levothyroxine treatment with impairment of regulatory T cells and emergence of stimulating autoantibodies. Additionally, there may be a switch in T cell populations due to possible preferential clearance of blocking over stimulating antibodies in pregnancy (6–8).

| Table 1. Serial thyroid function before and after levothyroxine dosing |
|---------------------------|---------------------------|
| TSH (mIU/L)               | 40.3–150            | 21.9           | 11.9          | 12.1      | 13.3     | 16.0     | 15.9      |
| T3 (nmol/L)               | 0.65–4.53           | 2.90           | 2.96          | 16.90     | 6.53     | 2.56     | 0.75      |
| auto-thyrotropin antibodies | 0–50                | <20            | <20           | <20       | <20      | <20      | <20       |
| TrAb (TBII) (IU/mL)      | <1.8                | >40            | <40           | <40       | <40      | <40      | <40       |
| Levothyroxine dosage     | 25                  | 37.5           | 50            | 50        | 50       | 50       | 50        |

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Accuracy of Direct Progesterone Immunoassay vs Liquid Chromatography Mass Spectrometry

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Background: Progesterone (P4) secreted by the corpus luteum is essential for implantation and early pregnancy. Serum P4 measurement on day of hCG administration during IVF controlled ovarian stimulation has been proposed to identify premature ovulation and/or luteinisation with an adverse impact on pregnancy in that IVF cycle.

Objective: To evaluate the accuracy of serum P4 measured by direct (unextracted) immunoassay (IA) vs a liquid chromatography mass spectrometry (LC-MS) reference method.

Method: Serum samples were collected from 254 women (median age 38, range 20-49 yr) on hCG day during an IVF cycle. Serum P4 was measured by IA (Beckman Coulter Access) and by LC-MS with results compared by Bland-Altman [BA], Passing-Bablok [PB] and Deming [D] regression methods. For analysis, left-censored (undetectable) results in LC-MS were assigned a value half of the detection limit (0.05 ng/ml).

Results: IA over-estimated serum P4 in every sample (median 4.8 vs 1.5 nM; median difference 4.4 nM [interquartile range 3.5, 5.9 nM]). Serum P4 was detected in 252 (99%) by IA and in 215 (85%) of samples by LC-MS. By PB regression, the intercept was 3.2 nM (95%CI 3.1, 3.3 nM) with a slope of 1.0 (95%CI 0.9, 1.1). By D, the intercept was 3.6 nM (95%CI 3.5, 3.8 nM). The upward bias of IA increased exponentially at low serum P4 concentrations (IA <5 nM or LC-MS<2 nM). Age was unrelated to either assay result or their difference.

Conclusion: IA consistently overestimates serum P4 levels so that low measurements (IA<5 nM) are too inaccurate to be used quantitatively. The utility of higher serum P4 measurements by IA and serum P4 and other steroids measured by multiplex LC-MS profiling in predicting IVF pregnancy outcomes warrants further investigation.
Paradoxical reduction in corticosteroid-binding globulin cleavage is seen in alpha-1 antitrypsin deficiency: implications for cortisol homeostasis

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**Background:** Corticosteroid-binding globulin (CBG) regulates the delivery of anti-inflammatory cortisol to tissues. High-affinity CBG (haCBG) is cleaved by the serine protease neutrophil elastase (NE) at sites of inflammation, resulting in permanent transition to low cortisol-binding affinity form (laCBG), releasing free cortisol. Alpha-1 antitrypsin (AAT) is the major circulating inhibitor of NE. Alpha-1 antitrypsin deficiency (AATD) is an autosomal codominant condition that predisposes patients to early-onset emphysema and cirrhosis due to increased proteolytic destruction from the inherent protease:antiprotease imbalance.

**Hypothesis:** That deficiency of AAT should lead to increased NE activity and therefore increased CBG cleavage *in vivo*, with decreased absolute and relative levels of the native haCBG and increased laCBG, with important implications for the pathogenesis and treatment of AATD.

**Methods:** We performed a prospective observational study of 10 patients with stable AATD and 28 controls. Plasma total CBG, haCBG and laCBG forms were measured by in-house parallel monoclonal ELISAs. AAT, total and free cortisol levels were also measured.

**Results:** Mean ± SEM circulating levels of total CBG were similar among AATD patients and controls (512 ± 46 and 498 ± 15 nmol/L; P=0.8), but haCBG was significantly higher (353 ± 38 and 264 ± 8 nmol/L; P<0.005), and laCBG lower (159 ± 19 and 225 ± 11 nmol/L; P=0.016) in the AATD group. The ratio of haCBG:totalCBG was significantly higher in AATD (69 ± 3% and 54 ± 1.3%; P=0.0001). There was a significant negative correlation between haCBG:totalCBG and AAT levels (P<0.05, R=0.67), but no correlation between AAT and cortisol indices.

**Conclusions:** Despite a lack of AAT and excess uninhibited NE, CBG cleavage is paradoxically reduced in AATD under basal conditions with increased absolute and relative levels of haCBG compared with controls. The pathogenic implications for cortisol delivery under conditions of acute or subacute infection require further study.

The Prevalence of BRAF V600 Mutations and its Associated Histopathology Features in Papillary Thyroid Carcinoma in New Caledonia and Australia

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New Caledonia (NC), a French territory in the Pacific, has the highest worldwide incidence of thyroid cancer1. We have previously shown a high prevalence of BRAFV600E mutation in this population in association with increased numbers of multifocal bilateral PTC. In this study, we aim to extend this study and to in a subset of BRAFV600E negative patients examine the incidence of other known BRAF mutations. Associations of these mutations with histopathological features were also examined.

The BRAF V600E mutation status was determined in 121 micro-dissected Formalin Fixed Paraffin Embedded (FFPE) PTC tumour tissue obtained from Laboratoire d'Anatomie et Cytopathologie, Nouméa, NC (n=49) and from RPA Hospital, Australia (n=72). BRAF V600E negative NC samples (n=15) were also examined for presence of BRAF V600Ec, V600R, V600D and V600K mutations. Pathological data were obtained from histopathology reports and patients’ medical records. Data was analysed by **Chi squared** analysis. In both populations, PTC was more common in females, similar to the pattern worldwide. BRAF V600E prevalence was 64% in NC and 55% in the Australian cohort and this mutation was significantly more common in NC multifocal bilateral tumours (NC: 92% vs Australian: 67%; P<0.005). The further screening for BRAF mutations in BRAFV600E negative samples from NC found that 13% presented BRAF V600Ec and 13% presented BRAF V600R (with no overlap between the two mutations). These incidences are higher than expected (4.3% and 4.9% respectively) for a given population. BRAF V600D and V600K mutations were not detected. The BRAFV600Ec mutation was only found in bilateral PTC and BRAF V600R only in unilateral PTC. The higher prevalence of the BRAFV600E and BRAFV600Ec mutation in the NC cohort with multifocal bilateral PTC may indicate more aggressive tumours in these individuals. Whether the NC population has increased incidence of other BRAF requires further investigation.
Effect of denosumab on glucose control in subjects with diabetes or pre-diabetes from the FREEDOM study

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High serum RANKL concentration was a predictor of incident type 2 diabetes (T2DM) in a population-based study, and blockage of RANKL signalling improved glucose intolerance by enhancing hepatic insulin sensitivity in mouse T2DM models (Kiechl et al. Nature Med 2013;19(3):358–366). Denosumab is a fully human monoclonal antibody that binds with high affinity and specificity to RANKL and prevents the formation, function, and survival of osteoclasts, and is associated with vertebral and nonvertebral fracture risk reduction. In a prior posthoc analysis of the FREEDOM trial, denosumab had no effect on incident diabetes or fasting serum glucose (FSG) in women without diabetes at baseline. Based on the favourable effect of RANKL blockage on glucose tolerance in mouse T2DM models, we hypothesised that denosumab decreases FSG in FREEDOM subjects with diabetes or prediabetes.

Baseline diabetes status was by self-report, use of antidiabetic medication (ADM), or an FSG ≥126 mg/dL on no ADM. Average postbaseline FSG across visits was estimated using a repeated measures model including treatment group, baseline FSG, BMI, and age; visit; ADM use; treatment-by-visit interaction; and ADM use-by-visit interaction as fixed effects.

Baseline characteristics were similar between denosumab and placebo in both diabetes and prediabetes subpopulations. Estimated average postbaseline FSG across visits was not significantly different between denosumab and placebo in women with either diabetes or prediabetes (p=0.20 and p=0.42, respectively); however, when censoring FSG values after ADM use in women with diabetes, estimated average postbaseline FSG across visits was lower with denosumab than placebo (p=0.02).

In this posthoc analysis, denosumab did not appear to affect FSG in subjects with diabetes or prediabetes. It remains to be determined whether blockage of RANKL has a clinically important effect on glucose metabolism.

Predictors For Surgically Resected Non-Functioning Pituitary Adenoma Requiring Secondary Intervention

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Background: Surgery is the primary mode of therapy for non-functioning pituitary adenomas (NFPA). The post-operative management of NFPA is a challenge because of a lack of knowledge regarding factors influencing remnant tumour growth that is clinically significant.

Aims: To identify radiological factors that predict the need for secondary intervention after surgical resection of NFPA.

Methods: This is a single-centre retrospective study of surgically resected NFPA in patients with pre- and serial postoperative MR imaging followed for at least a year. Tumour characterisation were performed by a single operator from pre-operative (tumour volume and extrasellar extension) and serial post-operative images (remnant volume, remnant site and growth rate). Secondary intervention was the outcome measure. The CVs for pre- and post-operative tumour volume from 8 subjects measured twice were 4% and 7% respectively.

Results: 85 patients (49 men, mean age at surgery: 53±16 years) with a median follow up of 5.1 years (range:1.2-20.0) were studied. The pre-operative median volume was 3447 mm³ (526-99850). Post-operatively, 67% had remnant tumours, 60% of which were extrasellar with a median remnant volume of 319 mm³ (33-5475) and remnant growth rate of 51.8 mm³/year (0-1963.2). 25% of patients required secondary intervention (second surgery: 8 and irradiation: 13). Kaplan-Meier analysis showed that the rate of secondary intervention when required was 65% at 5 years and 100% by 10 years. Cox regression analysis identified presence of post-operative remnant (HR: 5.1, CI: 1.6-11.2, p=0.01), remnant growth rate (HR: 3.3, CI: 2.1-7.0, p=0.01) and pre-operative suprasellar invasion (HR: 2.7, CI: 1.1-9.9, p=0.02) as independent predictors of secondary intervention.

Summary: In surgically treated NFPA, secondary intervention occurred in 25%, all within the first decade. This was determined by pre- and post-operative tumour characteristics.

Conclusion: In surgically resected NFPA, secondary intervention is unlikely to be required beyond 10 years (i) the presence of tumour remnant is the primary prognostic indicator (ii) intensity of follow up should be tailored to imaging characteristics...
Characteristics, Diagnoses and Clinical and Genetic Outcomes of Patient Population Attending a Multidisciplinary Familial Endocrine Neoplasia Clinic

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BACKGROUND
Heritable endocrine neoplasias include parathyroid and pituitary adenomas, phaeochromocytoma, paraganglioma and medullary thyroid cancer. Causative genes include RET, MEN1, NF1, VHL, SDHA, SDHB, SDHC, SDHD, SDHAF2, TMEM127 and MAX. As there are specific management guidelines for gene carriers1,2,4, appropriate screening of individuals is necessary. The Multidisciplinary Familial Endocrine Genetics Clinic was created to screen and manage affected patients.

METHODS
A retrospective audit of medical records was undertaken of all patients who had been referred to the Royal North Shore Hospital Multidisciplinary Familial Endocrine Neoplasia Clinic between April 2013 and May 2015. Patient characteristics and clinical and genetic diagnoses were assessed.

RESULTS
Sixty-eight new patients were referred, 21 (31%) male and 47 (69%) female. Age ranged between 12 to 83 years. The geographic referral area was predominantly across New South Wales, but also from the ACT and Queensland. Referral reasons included pre-existing paraganglioma (8, 11.7%), and phaeochromocytoma (7, 10.2%), affected family members (17, 25%), neuroendocrine tumours (4, 5.8%), medullary thyroid cancer (3, 4.4%), and adrenocortical cancer (3, 4.4%). Eleven asymptomatic individuals with an affected family member were diagnosed with a genetic mutation, 4 in SDHA, 6 in SDHB, one in SDHC). Genetic mutations in patients with paraganglioma and phaeochromocytoma include SDHA (n=3), SDHC (n=1), and pending (n=7) results. Genetic screening of four individuals with neuroendocrine tumours found one MEN1 gene deletion.

DISCUSSION
The spectrum of genetic mutations found in our audit are comparable to other studies: for instance, with SDH mutations accounting for 11%, and NF1 2% of the susceptibility genes in paraganglioma1. This clinic has facilitated identifying gene mutation carriers, who are being screened for phenotypic features, and this may reduce morbidity and mortality that would otherwise accompany delayed diagnosis.


Intermittent moderate energy restriction improves weight loss efficiency in diet-induced obese mice

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Intermittent severe energy restriction is an increasingly popular method of weight management. To investigate whether intermittent moderate energy restriction may improve this approach by enhancing weight loss efficiency, we conducted a study in mice, where energy intake can be unambiguously defined.

Male C57BL6 mice that had been rendered obese by ad libitum access to a diet high in fat and sugar for 22 weeks were then fed one of two energy-restricted normal chow diets for a 12-week weight loss phase. The continuous diet (CD) provided 82% of the energy intake of age-matched ad libitum chow-fed controls. The intermittent diet (ID) provided cycles of 82% of control intake for 5-6 consecutive days, and ad libitum intake for 1-3 days. Subsets of mice then underwent a 3-week weight regain phase involving ad libitum feeding.

Mice on the ID showed transient hyperphagia relative to controls during each 1-3-day ad libitum feeding period, and overall ate significantly more than CD mice (91.1 ± 1.0 versus 82.2 ± 0.5% of control intake respectively, n = 10, P < 0.05). There were no
Acromegaly: Outcomes from a single pituitary surgeon service in Christchurch New Zealand

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Background: Acromegaly is characterised by excess growth hormone secretion and is associated with increased morbidity and mortality. Current guidelines define cure or control as normal IGF-1 and random growth hormone concentrations <1μg/L (1).

Objective: To audit the immediate and long-term outcomes of patients treated surgically for acromegaly at Christchurch Hospital, New Zealand, a small tertiary referral centre with a single pituitary neurosurgeon.

Methods: We undertook a retrospective case review of all cases of acromegaly treated via endoscopic transnasal transphenoidal surgery between May 2000 and August 2013. Biochemical and clinical data concerning pre-operative findings, operative findings, and post-operative follow-up was collected.

Results: 40 patients (15 male, 25 female) were identified. 12 tumours were microadenomas, and 28 macroadenomas. All patients had at least one measurement of random GH and IGF-1 within 6 months of surgery (mean 44 days, range 2-105). 50% (6/12) of microadenomas met cure criteria compared with 35% of macroadenomas (10/28). Three patients with invasive tumours underwent stereotactic radiotherapy and 8 patients commenced medical therapy within 6 months of surgery.

Average follow-up was 70.1 months for 36/40 patients. 41% of patients were on medical therapy (octreotide, cabergoline or in combination), 50% of macroadenomas, 30% of microadenomas. 64% of patients had both IGF-1 and GH within target range; 54% of macroadenomas and 83% of microadenomas. 3 macroadenomas were controlled with cabergoline alone. 33/36 tumours had normal IGF-1. Mean random GH concentrations for macroadenomas was 0.90μg/L, for invasive tumours 1.66μg/L, and 0.56μg/L for microadenomas.
Conclusions: Surgical cure rates for microadenoma are lower than reported elsewhere in the literature but may not reflect true growth hormone status as many patients were assessed less than 3 months following surgery. Consistent measurement of growth parameters at least 3-6 months after surgery is recommended. Most patients achieved good biochemical control at long term follow-up although many require ongoing medical therapy. Cabergoline is an effective therapy even in patients with macroadenoma.


Graves' Dermopathy: a report of three cases

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Dermopathy is a recognized but rare extrathyroidal manifestation of Graves’ disease (GD), affecting 1.5% of patients. The pathogenesis of this manifestation remains poorly understood but is most likely triggered by autoimmunity to the thyroid stimulating hormone (TSH) receptor and possibly the insulin like growth factor (IGF-1) receptor. We present two cases of dermopathy related to GD to highlight the challenges associated with diagnosis and management of this condition.

The first case involves a 38 year-old man, diagnosed with GD in 1997. He was treated with carbimazole, followed by radioactive iodine. He then developed significant Graves’ orbitopathy (GO) requiring decompressive surgery. Following this he developed left great toe swelling with severe skin thickening, clubbing and erythema spreading up his left shin. Despite treatment with compression bandaging, lymphoedema dedicated physiotherapy, topical and intravenous corticosteroids his dermopathy progressed and now involves both lower limbs.

The second case involves a 53 year-old man diagnosed with GD in 2010. He had gross GO with proptosis, periorbital swelling, chemosis, lid lag and ophthalmoplegia. He also had clubbing and severe bilateral skin changes with circumferential involvement of his lower limbs, plaques, verrucous change and a 3x4cm soft tissue swelling overlying the proximal phalanx of his right great toe. He was treated with suppressive doses of carbimazole and with thyroxine replacement to maintain a euthyroid state. His GO and dermopathy have not improved despite intravenous methylprednisolone, topical steroid ointment and compressive bandaging.

Both patients have strong family history of autoimmune disease, extensive smoking history and consistently elevated TSH receptor antibodies despite treatment.

The mainstay of treatment for dermopathy is systemic glucocorticoid therapy however efficacy of this treatment is limited in severe disease. Multiple novel therapies are being investigated for GO, including rituximab, which may be applicable to treatment of dermopathy due to a likely shared pathogenesis.


Regrowth of non-functioning pituitary macroadenomas undergoing surgery in a single Australian centre.

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Non-functioning pituitary macroadenomas (NFPMA) are the commonest pituitary tumour requiring surgery. There are no published series regarding the surgical outcomes from Australia. We describe surgical outcomes and regrowth rate at a single centre.

Methods: Retrospective analysis of all NFPMA cases with pituitary surgery between September 1995 and December 2014.

Cohort: 178 cases identified. Males 54%, mean age 56.2±14.9 years.

Symptomatic presentation occurred in 61% (N=109) of which headache was the commonest complaint (N=69; 39%). Incidental presentation 29% (N=51); apoplexy in 10% (N=18). Visual deficit was reported in 67% (N=120).

Surgery:
The trans-sphenoidal approach was used in all except one who underwent the trans-cranial approach. Senior neurosurgeon (PMcN) performed 71% surgeries, the remainder were performed by five other neurosurgeons. A single operation occurred in 155 (87%). Two operations were performed in 20 (12%) and three in 3 cases (3%). In 23% (N = 6) repeat surgery was planned in the immediate post operative period. In 48% (N = 11) repeat surgery was performed at a mean follow up time of 55.3 months, no data on timing of repeat surgery in the remainder.

Post-operative complications: CSF leak (N=14; 8%), transient DI (N=27; 15%), permanent DI (N=12; 7%), SIADH (N=14; 8%), significant infection (N=3; 2%), significant bleeding (N=2; 1%), post-operative cardiac events (N=2; 1%).

Surgical Follow up:
One hundred and thirty-five patients (76%) had radiological follow-up ≥12 months, mean follow-up 81.8 (range 12-226). Thirty-three patients (24%) demonstrated tumour regrowth. Mean time to tumour regrowth was 59.7 months. Residual tumour was a significant risk factor for tumour regrowth (38% vs 15%; p=0.02). Treatment for tumour regrowth was surgery in 42% (N = 14), radiotherapy in 24% (N = 8) and combined approach in 15% (N = 5).

Discussion:
Tumour regrowth rate following trans-sphenoidal pituitary surgery is low, consistent with other international series.

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Examining the indications and results of bone densitometry performed in a large metropolitan teaching hospital.

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PROBLEM:
Osteoporosis is a condition associated with significant morbidity, mortality and economic costs. It is a disease amenable to primary and secondary prevention. The Medicare Benefits Schedule (MBS) is a list of Medicare services which are subsidised by the Australian Government. There are MBS criteria highlighting patients who would be eligible for investigation of osteoporosis with bone densitometry. The Pharmaceutical Benefits Scheme (PBS) is a part of the Australian Government’s National Medicines Policy, with the aim of providing access to necessary medicines for Australians through subsidising medication costs (pbs.gov.au). We suspect that there are patients referred for bone densitometry who do not meet the MBS criteria for investigation of osteoporosis. In addition, we are interested in examining the relationship between the bone densitometry results and the PBS criteria for prescription of osteoporosis treatments.

METHODS
A retrospective audit of patients who have undergone bone densitometry at the Lyell McEwin Hospital, South Australia, over a 6 month period, will be conducted. Data presented will include:

• Patient demographics
• The indication listed by the referring practitioner
• Whether this indication matches MBS listed indications for bone densitometry
• The result of the bone densitometry, including FRAX estimation

• How the bone densitometry result influences treatment


Post-Partum Osteoporosis Due To Systemic Mastocytosis: 2 Case Studies

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Mastocytosis is a rare cause of secondary osteoporosis. We present two cases of systemic mastocytosis being diagnosed in the setting of post-partum osteoporosis. Case 1: A 35 year old G2P2 woman who was breastfeeding presented with subacute on chronic back pain 4 weeks post-partum. Imaging confirmed the presence of multi-level vertebral fractures. T-score was -4.5 at the lumbar spine and -2.8 at the left hip. Vitamin D was 39nmol/L (N > 50), and calcium and PTH were not elevated. Screening tests for secondary osteoporosis revealed an elevated serum tryptase of 23.8ng/ml (N < 11ng/ml) and a subsequent bone marrow biopsy confirmed the presence of mastocytosis. When she was treated with a zoledronic acid infusion, she developed a sinus tachycardia, hypotension and a fever of 40°C. A recent report suggests that acute phase reactions may be a
common reaction related to the use of zoledronic acid in patients with mastocytosis (1). Case 2: A 29 year old G2P1 woman who was breastfeeding presented with acute on chronic back pain 3 months post-partum upon lifting her baby. Imaging confirmed a compression fracture of lumbar vertebrae 4-5. Her average T-score was -3.19 at the lumbar spine and -1.99 at the left hip. Her Vitamin D was 54nmol/L. She received calcium and vitamin D supplements. After a further 12 months there was only marginal improvement in her bone mineral density. Re-imaging revealed new compression fractures in the thoracic spine. Her serum tryptase level was elevated at 25.7 ng/ml and a diagnosis of mastocytosis was confirmed on bone marrow biopsy. She was commenced on an anti-histamine and has elected to have her osteoporosis treated with denusomab. Conclusion: Although pregnancy and lactation may contribute to bone loss, these cases suggest that in the setting of severe post-partum osteoporosis, a diagnosis of systemic mastocytosis should also be considered.


Hurt in the Sternum
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Publish consent withheld

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Desmopressin, Oxytocin and a Failing Heart
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A 27-year old female G2P1 presented at 33 weeks gestation with dyspnoea and peripheral oedema. Her past history consisted of diabetes insipidus (DI), thought to be nephrogenic, diagnosed at age 7, and obesity with a pre-pregnancy BMI of 53kg/m². Her mother, brother, uncle and 2 cousins were also affected by DI. She had not any endocrine review since childhood, and had maintained fluid balance by drinking 10L/day. She had not noticed any change in her fluid input or output during pregnancy. Following admission, investigations revealed a dilated cardiomyopathy (LVEF 28%), and a 2000ml/day fluid restriction was advised, posing a significant risk of dehydration and hypernatraemia given her unrestrained polyuria (>4.5L/day). A modified water deprivation test was performed with failure to adequately concentrate the urine at 4 hours, despite hyperosmolality (table 1). However, the urine osmolality increased following administration of desmopressin 1mcg. Subcutaneous desmopressin (1mcg bd) was commenced, allowing a modified fluid restriction to 3L daily with maintenance of normal serum sodium levels and stable fluid balance.

The patient developed acute pulmonary oedema and frusemide was commenced. Desmopressin was continued. Due to acute cardiac deterioration with SVT requiring adenosine, lower segment caesarean section (LSCS) was recommended at 37/40. Oxytocin was administered intraoperatively, but was not associated with any excess anti-diuretic effect (such as might occur with normal vasopressin responsiveness). Her newborn, however was noted to be hyponatraemic (Na 146-148mmol/L), with serum osmolality 320mosm/L and urine osmolality 100mosm/L suggestive of DI, and consistent with an autosomal dominant trait.

This patient’s management raised several challenges: the diagnosis of DI in pregnancy and the risks of dehydration; the response to vasopressin in nephrogenic DI; the need for fluid restriction in the presence of unrestrained polyuria; the potential impact of oxytocin on renal salt and water metabolism in nephrogenic DI.1,2
Table 1: Modified Water Deprivation Test

<table>
<thead>
<tr>
<th>Time</th>
<th>Baseline</th>
<th>4 hours</th>
<th>6 hours</th>
<th>8 hours</th>
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<tbody>
<tr>
<td>Serum Na (mmol/L) (Pregnancy Ref 132-140)</td>
<td>139</td>
<td>140</td>
<td>137</td>
<td>137</td>
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<tr>
<td>Serum Osmolality (mmol/Kg) (Pregnancy Ref 275-285)</td>
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<td>288</td>
<td>282</td>
<td>285</td>
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<tr>
<td>Urine Osmolality (mmol/Kg)</td>
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<td>242</td>
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<td>490</td>
</tr>
<tr>
<td>Serum Vasopressin (pmol/L) (Non pregnant Ref 0.1-7.0)</td>
<td>2.3</td>
<td>2.4</td>
<td>2.3</td>
<td></td>
</tr>
</tbody>
</table>

DDAVP 1mcg administered at time 4 hours (after blood and urine collection)
Patient resumed water intake at 4 hours
References ranges apply to pregnancy where specified.

A rare type of aggressive thyroid cancer: review of the literature for treatment options

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Ms KJ is 52 years old lady who presented with 6 weeks of subscapular and thoracic back pain. CT identified an osteolytic lesion and soft tissue mass in the thoracic spine, and an incidental left lobe thyroid mass causing contralateral tracheal displacement. MRI showed impending cord compression, necessitating a T5 vertebrectomy. Metastatic follicular thyroid cancer was diagnosed on histopathology.

Her thyroid ultrasound showed a left lobe thyroid nodule without clear tracheal invasion or lymph node involvement. Non-contrast CT demonstrated a low density mass with calcific foci replacing the left lobe of the thyroid gland. Lung metastases were not seen on X-ray, and her repeat MRI showed lesions consistent with haemangiomas.

A total thyroidectomy with lymph node resection was performed. Her left lobe had a 30x30x28mm tumour, containing a mixture of well and poorly differentiated regions. The differentiated areas demonstrated a follicular pattern with colloid filled microfollicles lined by atypical follicular cells, staining positive for thyroglobulin; the poorly differentiated areas had solid pattern of sheets and cords of tumour cells in a desmoplastic stroma without follicles, with increased staining for cyclin D1 and P63. No malignancy was found nodally. She was diagnosed with stage IV (T2N0M1) poorly differentiated insular variant of follicular carcinoma.

She was further treated with thyroxine withdrawal high dose radioactive iodine at 5300MBq. This reduced her thyroglobulin levels, although they remained elevated 3 months post (Fig.1). Combined PET and radioiodine scan (Fig.2) revealed new metabolically active but iodine inactive lesions in the liver and the right upper sternum, and a mildly iodine active but PET avid T5 vertebral body lesion. The spine was treated with radiotherapy, analgesia and dexamethasone. The liver lesion was confirmed to be a solitary metastasis on primovist MRI, which will be considered for surgical resection post radiotherapy.
Undiagnosed Asymptomatic Phaeochromocytoma Causing Intra-Operative Haemodynamic Crisis in a Patient with Type One Diabetes.

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A 41yo man with a background of type 1 diabetes was admitted with starvation ketosis and sepsis secondary to multiple necrotic soft tissue wounds obtained on a remote solo bush walk.

On presentation, the patient was alert and orientated. Initial tests showed hyperglycaemia with ketosis but normal acid base balance. Inflammatory markers were markedly elevated. Multiple scratches and cuts were noted as well as broad necrotic wounds on both feet, knees and right thigh as well as an infected right elbow bursa. Intravenous fluids, antibiotics and insulin infusion were commenced. The Plastic Surgical team arranged surgical washout and debridement that afternoon.

Induction of anaesthesia was complicated by low oxygen saturation and tachycardia. On insertion of the endotracheal tube, systolic blood pressure rose to 280mmHg. Esmolol 60mg was administered with no change in blood pressure. Medication error and arousal were excluded. A GTN infusion was commenced for the short procedure.

Post operatively; the patient was diaphoretic, febrile, tachycardic and hypertensive. Intravenous metoprolol was required over
the next 2 hours after which the patient was transferred to the intensive care unit. Differential diagnoses considered included septic shower or aspiration pneumonia.

Urgent plain film of the chest was normal. Contrast CT demonstrated an 8.2 x 6.8cm right adrenal mass prompting 24-hour urine catecholamines and plasma metanephrines, which were markedly elevated. Metaiodobenzylguanidine scan showed varying uptake at the periphery of the adrenal mass suggestive of pheochromocytoma, with no extra adrenal uptake. FDG PET detected no FDG avid disease, indicating low probability of high-grade adrenal malignancy.

Treatment was initiated with Phenylephrine and hyponatraemia was successfully treated. He was discharged from hospital on the first post admission day on 10% dextrose. Metoprolol was added on discharge. He was referred to endocrinology and further investigations were performed.

He was diagnosed with FD aged 4 and has extensive disease with marked craniofacial involvement, including right facial hemiatrophy, milia pigmentation, and may have gonadotropin-releasing hormone (GnRH) reserve. He has no evidence of ABC recurrence. Past complications include a fractured right femur and right optic nerve neuropathy requiring decompression surgery. He self-treated a second episode in 2013. He has no preoperative arterial ablation.

Histopathology confirmed a pheochromocytoma with no malignant features. Mutational analysis of the tumour showed normal staining for SDHB and SDHA.

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Hypoglycemic Encephalopathy and the Severity of Brain Injury: A Case Report

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Hypoglycemic encephalopathy is a potentially life-threatening event that can result in permanent brain injury. This syndrome is not well described in the literature. We report a case of hypoglycemic encephalopathy in a 33-year-old male with type 1 diabetes following a presumed accidental catastrophic insulin overdose. He was found unresponsive following a prolonged hypoglycemic period estimated to be 17 hours. Upon arrival, his blood sugar level (BGL) was too low to be recorded and his Glasgow Coma Scale (GCS) was 5. He was normothermic with a pH of 7.32 and had a lactate of 3.3 mmol/L. Despite rapid normalisation of his BGLs with 10% dextrose, he had minimal improvement in his GCS. He was intubated and transferred to the intensive care unit (ICU). A CT of his brain was suggestive of diffuse cerebral oedema. He progressed to a bilateral craniectomy to relieve his presumed raised intracranial pressures. Magnetic resonance imaging (MRI) of his brain performed day 6 post admission showed elevated T2 and flair signals throughout his cortex and elevated signal on the diffusion weighted imaging (DWI) was consistent with diffuse cytotoxic oedema. The basal ganglia was hyperintense on FLAIR and T2 images, however the thalami were spared. Reduced apparent diffusion coefficient (ADC) signal throughout the subcortical white matter was noted. He had minimal neurological improvements clinically and an electroencephalogram (EEG) showed very low voltage output in keeping with minimal cortical activity. In view of above findings, he was felt to have no prospect of recovery and was palliated. In summary, we report a case of severe hypoglycemic encephalopathy resulting in fatal metabolic brain injury that was difficult to prognosticate. The syndrome is associated with characteristic MRI findings as described in our case. We attribute prolonged hypoglycemia, normothermia and DWI findings as predictors of poor outcome in this case.

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A case of frontal bone aneurysmal bone cyst in association with polyostotic fibrous dysplasia

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We present the case of a 21 year old male who developed an aneurysmal bone cyst(ABC) on a background of fibrous dysplasia(FD). He was diagnosed with FD aged 4 and has extensive disease with marked craniofacial involvement, including ptuinary fossa. Past complications include a fractured right femur and right optic nerve neuropathy requiring decompression surgery. He self-treated a second episode in 2013. He has no hormonal abnormalities.

He subsequently developed a rapidly expanding left frontal bone lesion with imaging suggesting an ABC. Given the rapid expansion and his compromised vision, he underwent a craniotomy with excision of an 8x10cm lesion with minimal blood loss despite no preoperative arterial ablation. Histology showed a ABC arising from FD. Vision in the left eye is now completely normal. He has no evidence of ABC recurrence.

FD is an osteoblastic disorder in which bone is replaced by dysplastic fibrous tissue. FD is caused by a postzygotic activating mutation of the G-protein alpha-subunit. It can be monostotic or polyostotic, have overlying café-au-lait pigmentation, and may cause hormonal hypersecretion(McCune-Albright Syndrome). Malignant transformation, presenting with pain and an expanding mass, can occur, with polyostotic disease and previous radiation increasing risk. ABCs are rare benign lesions presenting with similar symptoms but distinct features on imaging. ABCs can be either primary or secondary to malignancies or FD. There are several theories for the pathogenesis of ABCs. Treatment is surgical with a high risk of intraoperative haemorrhage. The combination of craniofacial FD with secondary ABC is rare with limited cases in the literature.

Our case is of a 21 year old male with FD who develops an ABC. We review the literature in regards to craniofacial FD, ABC, treatments and outcomes.

MEN1 and paraganglioma: expanding the clinical spectrum of MENIN mutations.
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Multiple endocrine neoplasia type 1 (MEN1) classically consists of parathyroid, pituitary and pancreatic tumours. Here we report two unrelated cases with MEN1 with asymptomatic parangliomas.

P1 had a strong family and personal history of MEN1. Given the presence of pancreatic lesions with mild elevation of gastrin, a 18F-DOTATATE scan was undertaken. Unexpectedly this demonstrated uptake in the left carotid region suggestive of paranglioma. P1 had no symptoms or biochemical evidence of catecholamine excess. Histology of the resected mass showed a paranglioma with weak but positive staining for SDHB unlikely to be consistent with germline SDHx mutations.

P2 also had a strong family and personal history of MEN1. Although biochemically stable, he had an increasing pancreatic mass (>3cm diameter) with marked uptake on 18F-DOTATATE. FDG-PET suggested a high grade/poorly differentiated lesion and he underwent a Whipple's resection. Histology demonstrated a Grade 1 neuroendocrine tumour (35mm diameter) and a second lesion (8mm diameter) consistent with an extraadrenal paranglioma that stained positively for SDHB. Germline screening thus unlikely).

Germline screening of all exons of MENIN showed that P1 was heterozygous for a c.1716delC mutation in exon 10, resulting in a frameshift and introduction of a premature stop codon. P2 was heterozygous for a c.1319delG mutation (exon 9), with similar effect.

Sanger sequencing of DNA extracted from each tumour demonstrated loss of wildtype allele. Microarray genotyping (assessing for large copy number alteration) demonstrated loss of heterozygosity of chromosome 11 in both tumours, including the MENIN locus. Of note, there was differential aneuploidy of the paranglioma and adjacent islet cell tumour in P2.

The combination of paranglioma in MEN1 has been reported extremely rarely (four cases). P1 and P2 are undergoing germline screening for known phaeochromocytoma/paraganglioma susceptibility genes. However, if negative, our data suggest that paranglioma may rarely be part of the MEN1 syndrome.

Tetany Associated with Teriparatide Therapy: A Case Report
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Teriparatide is a parathyroid mimetic used in the treatment of severe osteoporosis to increase bone mineral density. Hypercalcaemia is a documented potential adverse effect. We present a unique case in which symptomatic hypocalcaemia and hypomagnesaemia followed initiation of Teriparatide therapy.

PT, a 30-year-old Cambodian female presented to the emergency department with symptomatic hypocalcaemia following commencement of Teriparatide for severe osteoporosis deteriorating despite antiresorptive therapy. Other medical issues included autoimmune hepatitis (cirrhosis and portal hypertension), very low weight (BMI 13.8kg/m2), secondary amenorrhoea, anaemia and intermittent electrolyte and mineral disturbances (hypokalaemia and hypomagnesaemia). Serum electrolytes and minerals prior to commencing Teriparatide were essentially within normal limits.

Following nine days of initial Teriparatide therapy, PT developed tetany and presented to the emergency department. Her ionised Calcium was 0.99mmol/L, corrected Ca2+ 2.0mmol/L, Mg 0.5mmol/L, PO4 0.85mmol/L, and renal function was normal. Liver function tests were elevated but not significantly different to the patient’s usual levels. A repeat Vitamin D was borderline at 50nmol/L. PT was closely monitored and stabilised with intravenous magnesium, and discharged on calcitriol 0.25mcg daily, magnesium supplementation and ongoing Teriparatide therapy.

Teriparatide is an anabolic bone formation agent, comprising an active fragment of endogenous human PTH and is known to be associated with transient post-dose hypercalcaemia (>2.6). There is currently no literature that has demonstrated Teriparatide being linked with hypocalcaemia or hypomagnesaemia. In fact there is emerging evidence of Teriparatide being used as a treatment for hypoparathyroid-associated hypocalcaemia. It is postulated that Teriparatide may have a converse effect in vulnerable individuals.

This case is the first of its kind in the literature and realises the potential for Teriparatide to cause hypocalcaemia. Given the severity of symptoms, early detection is essential to prevent significant complications.

Bilateral macronodular adrenal hyperplasia and systematic testing for aberrant receptors: a bumpy journey

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Mrs SD is a 62-year-old Bosnian refugee, incidentally discovered to have bilateral nodular adrenal enlargement during investigation for haematuria. She had no specific examination features of Cushings’s syndrome but was centrally obese and had a history of type 2 diabetes, ischaemic heart disease, hypertension, stroke and osteopenia. Cortisol failed to suppress after low and high dose dexamethasone and although incompletely suppressed, ACTH was low on repeated assessments, consistent evidence that circulating hormones other than ACTH stimulate adrenal cortisol production via ectopic or deviant eutopic receptors for these hormones on adrenocortical cell membranes. Blockade of these receptors has resulted in variable success as therapy for Cushings’s syndrome.

Bilateral macronodular adrenal hyperplasia (BMAH) is a rare cause of Cushings’s syndrome and usually presents around the fifth decade of life. In BMAH, there is emerging evidence that circulating hormones other than ACTH stimulate adrenal cortisol production via ectopic or deviant eutopic receptors for these hormones on adrenocortical cell membranes. Detection of aberrant receptor(s) can be achieved via targeted stimulation with potential candidate hormones. Using a protocol developed by Lacroix5, a strongly positive response to vasopressin was demonstrated; baseline cortisol rose by 119% without significant change in ACTH. A cortisol rise of 39% was also observed with Metoclopramide.

No cortisol rise was observed following subsequent testing with Desmopressin, a V2-selective agonist. The initial cortisol surge was thus thought due to aberrant V1 receptors. Aberrant V1 and SH-4 receptors are reported to be common causes of adrenal Cushings’s syndrome in BMAH. Detectable bioactive ACTH was recently demonstrated in adrenal tissues of BMAH patients5. Hormones implicated with aberrant receptors also stimulated ACTH production by these adrenal explants. These in vitro findings raise the possibility that steroidogenesis in BMAH is not ACTH-independent, as previously supposed. This may explain why ACTH was incompletely suppressed.

This case raised our awareness of steroidogenesis by aberrant receptors in adrenal Cushings’s syndrome and challenged the paradigm of ACTH-independent Cushings’s syndrome.

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Multiple paragangliomas in a 17-year old male with post-micturition symptoms

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A 17-year old man presented with palpitations, headache and diaphoresis after micturition and macroscopic haematuria. His plasma normetadrenaline levels were grossly elevated (15000 pmol/L) indicative of a paraganglioma. His paternal uncle had a mediastinal paraganglioma at age 22 and his paternal grandfather had a renal cell carcinoma at age 70. Computed tomography (CT) scans showed a bladder wall tumour, para-aortic mass, right hydrenephrosis, and an 8 mm left lung base lesion. A 18F-fluorodeoxyglucose (FDG) positron emission (PET) CT scan confirmed FDG avid lesions in the bladder, the pelvis and the aorto-caval region. Only the latter was clearly visible on 123I-metaiodobenzylguanidine scintigraphy. After pre-operative alpha- and beta-adrenergic receptor blockade, right ureteronephrectomy, partial cystectomy and resection of the aorto-caval and para-aortic vessel masses was performed. Histopathology confirmed multiple paragangliomas. There were no tumour-positive lymph nodes and immunohistochemistry staining for succinate dehydrogenase (SDH) B was absent, suggesting a germline mutation in either the SDHB, SDHC or SDHD gene. He is booked for a post-operative gallium PET scan to assess presence of residual tumour masses.

Bladder paraganglioma is a rare form of paraganglioma. One-third of patients with a phaeochromocytoma or paraganglioma are thought to have a germline mutation in one of the known susceptibility genes. In this case, a hereditary cause is strongly
suspected because of his young age, the presence of multifocal disease and a positive family history. Which genetic mutations to test for depends on tumour location, biochemical profile and immunohistochemistry. Genetic counselling is warranted once a germline mutation has been confirmed.

### Hemiballismus: a rare complication of diabetic nonketotic hyperosmolar state

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A sixty-three year old female with a 13-year history of type 2 diabetes treated with oral agents alone presented with sudden onset of left-sided hemiballismus. She had omitted her treatment for a number of months prior to presentation and HbA1C was 14.9%. A magnetic resonance imaging (MRI) scan of her brain showed a high signal on diffusion-weighted and hyperintensity on T1 weighted images in the right medial lentiform nucleus and head of caudate. Blood tests indicated severe hyperglycemia (serum glucose 26.2 mmol/L). She was diagnosed with hyperglycemia induced chorea-ballismus (HICB). After prompt treatment of her hyperglycemia with insulin, her hemiballismus resolved completely within 10 days.

HICB is a rare complication of hyperosmolar hyperglycemic state (HHS). It is characterized by a sudden onset of uni- or bilateral choreatic or ballistic movements in the context of severe hyperglycemia. There is a predilection for elderly women and occurs more frequently in Asians, suggesting a genetic susceptibility. Radiologically, HICB is associated with high signal intensity in the basal ganglia on T1 weighted sequences with the putamen being most frequently affected. Several mechanisms have been suggested including hyperglycemia-induced depletion of cerebral gamma-aminobutyric acid, activation of inflammatory cascades and regional hypoperfusion as a result of increased cerebrovascular resistance and hyperviscosity. However, the pathophysiology remains elusive. Treatment of the hyperglycemia results in quick resolution of symptoms in most cases. In patients presenting with unexplained hemiballismus, hyperglycemia should be considered as it is an easily treatable cause leading to quick recovery if treated promptly.

### Euglycaemic diabetic ketoacidosis in a young adult with type 1 diabetes and an eating disorder

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3. University of New South Wales, Sydney, NSW, Australia

A 17 year old female with a 3 year history of type 2 diabetes treated with oral agents alone presented with sudden onset of left-sided hemiballismus. She had omitted her treatment for a number of months prior to presentation and HbA1C was 14.9%. A magnetic resonance imaging (MRI) scan of her brain showed a high signal on diffusion-weighted and hyperintensity on T1 weighted images in the right medial lentiform nucleus and head of caudate. Blood tests indicated severe hyperglycemia (serum glucose 26.2 mmol/L). She was diagnosed with hyperglycemia induced chorea-ballismus (HICB). After prompt treatment of her hyperglycemia with insulin, her hemiballismus resolved completely within 10 days.

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**Euglycaemic diabetic ketoacidosis in a young adult with type 1 diabetes and an eating disorder**

**Angela S Lee**, Tang Wong, Jeff R Flack

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2. Sydney Medical School, University of Sydney, Sydney, NSW, Australia
3. University of New South Wales, Sydney, NSW, Australia

A 17 year old female presented to our young adults clinic in healthcare transition from paediatric endocrinology. She had a 12 year history of poorly controlled type 1 diabetes (T1DM) (recent HbA1c 18.2%), and a 3 year history of restrictive-type eating disorder. Her diabetes treatment was a basal-bolus insulin regimen, but she intentionally omitted doses to facilitate weight loss. She did not complain of feeling unwell and denied recent acute illness. She weighed 14.9kg (BMI 18.4kg/m²). On examination, her blood pressure was 120/60mmHg (no postural drop), pulse 118bpm (sinus rhythm), respirations 18 breaths/minute, and she was mildly dehydrated clinically. Capillary glucose was near normal at 9.9mmol/L, however fingerprick ketones were significantly elevated to 6.0mmol/L. Venous blood gas confirmed a metabolic acidosis with pH 7.18 (subsequently calculated to be high-anion gap).

Her diagnosis was euglycaemic diabetic ketoacidosis in the setting of chronic starvation and insulin omission, on a background of T1DM and restrictive-type eating disorder.

She was admitted to ICU for intravenous normal saline, dextrose, insulin infusion and electrolyte replacement, with resolution of T1DM and eating disorder over 24 hours. She is receiving ongoing care for her T1DM and eating disorder through a multidisciplinary team approach involving the endocrinologist, diabetes educator, dietitian, psychologist and psychiatrist.

**Teaching points:**
- While diabetic ketoacidosis (DKA) is generally defined as the triad of hyperglycaemia (blood glucose>11.0mmol/L), ketosis and metabolic acidosis, it can also occur rarely with near-normal glucose levels.
- Euglycaemic DKA can occur in people with T1DM and chronic starvation.
- A high index of suspicion is required as presentation may include minimal acute symptoms.
- Individuals with T1DM have a higher prevalence of dysfunctional eating behaviours and overt eating disorders. The care of these people with dual diagnoses can be highly challenging, and optimally requires a multidisciplinary team approach.
- This case highlights the value of point-of-care blood ketone assessment.
Double Trouble In The Pituitary: A Case Report
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Most cases of acromegaly are due to pituitary somatotroph adenomas, however a minority (<2%) of cases are due to GHRH hypersecretion (1). Mixed pituitary adenoma and gangliocytoma tumours are rare, and less than 80 cases are described in the literature (2). Most intra-pituitary gangliocytomas are associated with hormonal hypersecretion, commonly growth hormone (GH) excess (2). We report a case of acromegaly secondary to a mixed pituitary adenoma-gangliocytoma, and discuss the possibility of ectopic GHRH secretion from gangliocytomas.

A 60 year old male was referred for assessment of a pituitary mass found following investigation of chronic headaches over the preceding two years. MRI head revealed a 1.9 x 1.7 x 2.4cm right sided pituitary macroadenoma with invasion into the right cavernous sinus but no compression of the optic chiasm.

Examination findings were consistent with acromegalic features. He had no other symptoms or signs of endocrine dysfunction, nor family history of endocrinopathies. Static pituitary hormone testing showed an elevated IGF-1 122 nmol/L and elevated GH 5.2 ug/L. The remaining pituitary hormonal profile was normal. His GH failed to suppress following an oral glucose tolerance test (OGTT) (GH nadir 3.1 ug/L).

He underwent uncomplicated endoscopic trans-sphenoidal resection of the mass. Histopathology revealed a gangliocytoma (composite chromophobe pituitary adenoma and ganglion cells in neutropil). Immunohistochemistry of adenoma cells stained weakly for GH. Immunostaining for GHRH has been requested. An ultra-early (day 2) post-operative OGTT demonstrated suppression of GH to <1ng/ml. This will be repeated at 8-12 weeks post-operative.

In conclusion, we report an uncommon case of a mixed pituitary adenoma-gangliocytoma causing acromegaly. We hypothesise that ganglion cells secrete GHRH, subsequently inducing GH secretion from the adenoma cells. We review the literature to see if these lesions behave differently to classic acromegaly.


An unusual cause of recurrent severe hypokalaemia
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Context: Small cell prostate cancer has rarely been reported in associated with ectopic secretion of adrenocorticotropic hormone (ACTH) and severe clinical Cushing's syndrome.

Case description: A 91 year old man presented with hypertension and peripheral oedema. His background history consisted of hypertension, type 2 diabetes, atrial fibrillation, transient ischaemic attack, and prostate carcinoma with resection. On examination he had upper limb bruising, centripetal obesity and moderate pitting oedema. He was found to have a metabolic alkalosis, hypokalaemia (K+ 2.4 mmol/L) and initially received intravenous potassium followed by oral replacement. Investigations revealed markedly elevated morning serum cortisol and ACTH, and non-suppression on a 1mg dexamethasone suppression test. Imaging of his brain, chest, abdomen, and pelvis were normal. Further evaluation of the presumed ectopic secretion of ACTH was not undertaken because of the frailty of the patient and his clearly expressed wishes. Bilateral adrenalectomy was also considered but declined.

Management consisted of ongoing potassium replacement and Ketoconazole 400mg/day commenced with the aim of inhibiting cortisol synthesis. This led to an improvement in serum potassium but was poorly tolerated. Metyrapone 500mg/day was also trialled but ceased due to the development of abdominal pain and diarrhoea. There was little improvement in overall health and the patient opted for medication withdrawal. Post mortem examination revealed high-grade small cell prostate carcinoma, which is a very rare cause of ectopic ACTH.

Conclusions: In difficult to treat hypokalaemic alkalosis the differential diagnosis of ectopic ACTH Cushing's syndrome should be considered. Whilst most causes of ectopic ACTH secretion are found within the chest it is important to contemplate other aetiologies such as prostate cancer.
Pituitarius, where art thou?
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Publish consent withheld


Flushed with excitement – a heartfelt case of carcinoid syndrome
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Neuroendocrine tumours (NETs) are a heterogeneous group of neoplasms that arise from neuroendocrine cells of the gastrointestinal tract. Carcinoid syndrome with the classic triad of flushing, palpitations and diarrhea/abdominal pain, is more specifically attributed to well-differentiated serotonin-secreting midgut tumours and is present in approximately 20% of NETs in the duodenum and jejunum¹⁻². Individuals with carcinoid syndrome can present with chest tightness or breathlessness³ leading to diagnoses of acute coronary syndrome or asthma. These symptoms are thought to be related to serotoninergic activity of the NETs. Research suggests an association between the increased serotonergic activity⁴⁻⁵ with Takotsubo cardiomyopathy⁶, a transient cardiac syndrome with left ventricular hypokinesis or dyskinesis⁶.

We present the case of Ms LT, a 64-year old woman who presented with palpitations and chest pain in the setting of intense emotional stress, with dynamic ECG changes and troponin rise. This is on a background of multiple pulmonary embolisms, late-onset asthma and hypertension. Her family history is significant for Graves’ disease and the death of her father from a pancreatic tumour. Coronary angiogram showed pristine coronary arteries with apical ballooning and MRI showed basal inferior hypokinesis, normal contractility leading to a diagnosis of Takotsubo cardiomyopathy.

She represented one month later with PR bleeding, abdominal pain, diarrhoea, flushing and refractory palpitations. Investigations showed a neuroendocrine tumour of the ileal region with lymphatic and bony metastases. Chromogranin A and 24-hr urinary 5HIAA were elevated, however, after ceasing her proton-pump inhibitor her Chromogranin A normalised. She underwent surgical resection of her ileal tumour and was commenced on somatostatin analogue, which is keeping her carcinoid syndrome under control.

Increased plasma serotonin has been reported in Takotsubo cardiomyopathy. Clinical suspicion of carcinoid syndrome in a patient with Takotsubo cardiomyopathy should be raised with the presentation of the classic triad: flushing, palpitations and diarrhoea/abdominal pain.

Thyroid hormone resistance, a case report

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Introduction
Thyroid hormone resistance is a rare but important differential to consider in patients with hyperthyroxinaemia. The clinical presentation is that of non-suppressed thyroid stimulating hormone (TSH), elevated thyroid hormone levels and goitre with minimal clinical symptoms of thyrotoxicosis. The differential diagnosis for this hormone profile is TSH secreting pituitary adenoma.

Case Report
A 31-year-old woman presented with long standing deranged thyroid function tests in the setting of a strong paternal family history of thyroid disease.

At the time of initial presentation at age 15, she had a goitre and markedly elevated triiodothyronine (T3) (16.3pmol/L) and thyroxine (T4) (42.7pmol/L) levels with a non-suppressed thyroid stimulating hormone (TSH) level (1.79 mU/L). A computed tomography (CT) study of her brain, performed in lieu of magnetic resonance imaging (MRI) due to claustrophobia, did not demonstrate a pituitary adenoma. She went on to have a thyrotropin releasing hormone (TRH) test (200ug IV TRH), which demonstrated an appropriate rise in TSH (12.34mU/L at 20 minutes, 10.41mU/L at 30 minutes).

At the age of 26 she underwent a total thyroidectomy, complicated by transient hypoparathyroidism. Thyroid histology showed diffuse hyperplasia but no lymphocytic infiltration. She has subsequently required thyroxine replacement, with varying doses. She has a significant family history for thyroid disease, affecting multiple primary and secondary relatives on her father’s side.

Discussion
Thyroid hormone resistance is a rare autosomal dominant condition involving a mutation of the thyroid hormone receptor beta gene. It is estimated to occur in 1 in 40,000 live births. These patients have resistance to thyroid hormone in peripheral tissues. Variability of peripheral resistance means patients can have mixed clinical features of both hyper and hypothyroidism. These patients generally require supraphysiological replacement doses of thyroxine to achieve a relatively euthyroid state with TSH suppression.

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Vitamin C deficiency: an overlooked risk factor for impaired wound healing in patients with diabetes mellitus

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Vitamin C deficiency is rarely diagnosed in the modern era. With the Australian population eating more discretionary food and inadequate vegetables, it is possible that Vitamin C deficiency is becoming more prevalent. Groups with a greater tendency to avoid certain foods are at risk of developing manifest scurvy.

A 25 year old male with a lifelong history of Type 1 diabetes and a 10-year history of Coeliac disease attended Diabetes Clinic with multiple lesions on the anterior lower limbs. He stated they resulted from having dropped sheet metal on his legs three days prior. He was admitted with hyperglycaemia and non-acidotic ketosis, weight loss of 29% (22kg) over 6 months and microcytic anaemia. Vitamin C deficiency was suspected after dietary history revealed irregular compliance with gluten-free diet and minimal intake of fresh fruit and vegetables. Low vitamin C level was confirmed at 19 umol/L (normal > 40).
Antibiotics, oral vitamin C, vitamin D and iron supplementation were commenced. Psychiatry review excluded disordered eating. After discharge he stopped taking vitamin supplements and his vitamin C level was not replete at 30 umol/L. His leg wounds remained open but not infected. Two months later he lost a further 3kg weight, suffered postural dizziness and his leg wounds still had not healed. His vitamin C level was 35 umol/L with intermittent adherence to oral replacement. He started consistently taking 2000 mg daily and vitamin C level improved to 181 umol/L one month later. His wounds healed despite ongoing poor glycaemic control (HbA1c 12.2% from 11.7% previously).

Conclusions:
Although this patient had several potential factors contributing to his non-healing wounds, only achieving adequate Vitamin C replacement correlated temporally with wound healing. Vitamin C deficiency should be considered in patients with diabetes and non-healing wounds as the treatment is simple, affordable and safe.

Addition of a Mitochondrial Antioxidant to Culture Media Improves Embryo Development and Metabolism in an Aged Mouse Model

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Women are delaying starting a family and as a result the age of first time mothers has increased. The number of women >40yrs using IVF reproductive technologies has also increased. However, IVF is substantially less effective for women over 40 years of age. Recent data has established that mitochondrial function in eggs from older women is reduced and levels of reactive oxygen species (ROS) are increased. The aim of this study was to establish if the addition of an antioxidant which targets mitochondrial produced ROS, can improve embryo metabolism and development using an aged mouse model.

Zygotes were collected from 22 week old superovulated C57BL6 females and cultured at 37°C in 6%CO2:5%O2:89%N2 in either (i) control media (G1) or (ii) G1 + 100µM Manganese(III) tetrakis (4 benzoic acid) porphyrin (MnTBAP) which can traverse the inner mitochondrial membrane and neutralise superoxide anions. Embryos were transferred to G2 medium before assessment for on-time blastocyst development and glucose uptake (day 4- total of 74h culture) and cell number and differentiation (day 5- total of 91h culture) and ROS production (MitoSox).

Embryos cultured in the presence of MnTBAP were significantly more advanced on day 4 with higher levels of on-time blastocyst development (control 19.5%, MnTBAP 32.7%; P<0.05) which coincided with a 12% increase in glucose uptake (P<0.05) and a significant reduction in ROS production (-25.3%; P<0.01). There was a significant increase in cell number of the blastocysts cultured with MnTBAP (control 60.5±4.6, MnTBAP 73.1±3.7; P<0.05), which is usually indicative of increased viability. This increase was confined to the oxidative trophectoderm cells (control 43.4±3.7, MnTBAP 57.3±3.3; P<0.05).

This study indicates that embryo development of embryos from older mothers can be improved by the addition of a mitochondrial antioxidant. Assessment of pregnancy outcomes from these embryos is required to further validate these findings.

Decreased antioxidative gene expression in skeletal muscle in individuals conceived by In-Vitro Fertilisation (IVF)

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Objective
We have previously shown that adults conceived by IVF were more insulin resistant than naturally conceived adults (controls). However, the underlying mechanisms are not clear. This study aimed to compare circulating inflammatory cytokines and expression of genes involved in oxidative stress in skeletal muscle and subcutaneous adipose tissue.
Materials and Methods
Adults conceived by IVF (n=14) and controls (n=20) matched for age (17–26 years), gender, weight and body fat composition were studied. Subjects were examined after three days of an energy balanced diet (30% fat, 15% protein, 55% carbohydrate) and again after 3 days of overfeeding (+1250 kcal/day, 45% fat, 15% protein, 40% carbohydrate). Vastus lateralis muscle and abdominal subcutaneous adipose tissue biopsy samples were obtained from 6 IVF and 11 controls. Serum levels of IGF1, Adiponectin, C-reactive protein (CRP) and monocyte chemotactic protein-1 (MCP-1) were examined by ELISA. Markers of oxidative stress (superoxide dismutase (SOD) 1 and 2, glutathione peroxidase 1 (GPX1) and catalase) were measured by qPCR in both tissues.

Results
There was no difference between groups in serum levels of IGF1, adiponectin, CRP and MCP-1, independently of diet. At baseline, relative gene expression of SOD2 and GPX1 in skeletal muscle was significantly lower in IVF adults versus controls (P=0.02 and 0.04 respectively).

Conclusions
The data shows that inflammatory mediators were not altered in young IVF adults, although the expression of enzymes involved in antioxidative SOD2 and GPX1, were significantly lower. Further studies are warranted to determine if oxidative stress contributes to peripheral insulin resistance observed in IVF individuals.

Efficacy of a Cue-Mate Intravaginal Insert and Injection of Prostaglandin F2α For Synchronizing Estrus in Hanwoo Cattle
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Publish consent withheld

Epigenetic and microRNA-mediated regulation of adult hippocampal VGLUT2 expression following early prenatal ethanol exposure
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Maternal consumption of alcohol during pregnancy is associated with structural and functional abnormalities of the central nervous system in the offspring however the underlying mechanisms are not fully understood. We used an inbred C57BL/6J mouse model of early gestational ethanol exposure that is equivalent, developmentally, to the first 3-4 weeks of pregnancy in humans to examine the long-term consequences on gene expression and epigenetic state in the hippocampus. Solute carrier family 17 member 6 (Slc17a6), which encodes vesicular glutamate transporter 2 (VGLUT2), was significantly up-regulated in the hippocampi of adult ethanol-exposed male offspring. Transcriptional activation was associated with changes in both promoter DNA methylation and histone H3 lysine 4 trimethylation, a mark of active chromatin. Several ethanol-sensitive microRNAs were also identified in the hippocampus, one of which was shown to specifically interact with Slc17a6, revealing an additional level of post-transcriptional control. A significant correlation between microRNA expression in the hippocampus and serum of ethanol-exposed offspring was also observed.

Prenatal ethanol exposure has complex transcriptional and post-transcriptional effects on Slc17a6 (VGLUT2) expression in the mouse hippocampus. Altered epigenetic and microRNA-mediated regulation of glutamate neurotransmission in the hippocampus could contribute to the cognitive and behavioural phenotypes observed in fetal alcohol spectrum disorders. Our results also support the idea that circulating microRNAs could be used as biomarkers of early gestational ethanol exposure and/or hippocampal dysfunction.

Factors contributing to the poor reproductive performance of ewe lambs
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The majority of ewes in New Zealand are bred for the first time as 2-tooths when they are approximately 18-20 months old. Mating ewe lambs so that they produce a lamb at one year of age (yearlings) provides a clear opportunity to improve farm profitability through increasing the lifetime production of each ewe. If ewes lamb, on average, 4 times during their lifetime, producing a litter in their first year of life (so that they lamb 5 times), has the potential to increase their lifetime production by 25% thereby improving efficiency.
On average, a ewe lamb put to the ram will produce only 0.6 lambs to weaning, compared to an average of 1.2 lambs in older ewes. If the preferential feeding required to successfully lamb yearlings is also considered, the potential gain can be quickly eroded. Understanding the cause of this low efficiency and designing methods to improve or mitigate these effects would likely increase adoption of lambing yearlings.

We examined two cohorts of ewes selected over 2 years and mated in order to lamb at 1 and 2 years of age. Onset of puberty, ovulation rate, mid-pregnancy litter size, number of lambs born and number of lambs weaned were measured. Lower ovulation rate (1.54±0.07 vs 2.00±0.09), failure to be mated by the fertile ram and lower embryo survival (66% vs 78%) contributed to the poor reproductive efficiency of the younger animals. In a second study, the mid-pregnancy litter size of ewes mated in order to lamb at 1 year of age was shown to be greater in those ewes with a higher ovulation rate suggesting that manipulation of ovulation rate could be used to improve the efficiency of lambing yearlings.

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Differential maternal and paternal genome effects on circulating thyroid hormone concentrations and deiodinase expression in the midgestation fetus

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Deiodinases in placental and fetal tissues regulate the bioavailability of thyroid hormones and thus play an essential role in fetal growth. Non-equivalence of maternal and paternal genomes, i.e., genomic imprinting, has been demonstrated in the thyroid axis1. Deiodinase 3 (DIO3) is expressed from the paternal allele only and converts thyroxine (T4) to reverse triiodothyronine (rT3), preventing overexpression of fetal tissues to triiodothyronine (T3). DIO1 and DIO2 on the other hand, convert T4 to T3. Genomic imprinting and other epigenetic mechanisms that lead to allelic imbalance can impact on estimated effects of genetic markers involved in thyroid hormone regulation. Parental genome effects on fetal thyroid hormone levels and deiodinase expression have not been studied. We have previously demonstrated that a bovine model with Bos taurus taurus and Bos taurus indicus genetics in purebred and reciprocal cross fetuses at midgestation (Day153) can dissect maternal and paternal genome effects on fundamental fetal characteristics1. Here we show in the same resource (n=73) using linear models, that paternal genome affects umbilical cord plasma rT3 (P<0.001) and total T4 (P<0.001) levels, while maternal genome affects cord plasma free T4 (rT4) (P<0.001). Circulating T3 and rT3 levels were below assay sensitivity. Hepatic DIO1 transcript abundance was affected by maternal genome (P<0.001) and correlated with plasma rT4 (r=0.28, P<0.05) and rT3 (r=0.26, P<0.05). Renal DIO3 transcript was correlated with plasma rT4 (r=0.45, P<0.001) and rT3 (r=0.29, P<0.05), but was not affected by paternal genome. Consistent with imprinted paternal expression, placental DIO3 transcript was affected by paternal genome (P<0.05), but was not correlated with circulating hormone, suggesting it is not a major contributor of fetal rT3. In conclusion, we have demonstrated strong differential parental genome effects on thyroid hormones and correlated these with major regulators of thyroid hormone metabolism.


Consequences of culturing preimplantation embryos individually

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IVF clinics increasingly culture embryos individually to facilitate morphometric and genetic analyses. However, culturing embryos individually rather than in groups deprives them of paracrine factors produced by neighbouring embryos and this reduces blastocyst cell numbers. Further examination of the differences between embryos cultured individually or in groups, including their response to other stresses in vitro and the role of paracrine factors, is therefore warranted.

Zygotes from CBA x C57BL/6 mice were cultured individually in 2 µL G1/G2 medium or in groups of 10 in 20 µL at 5% oxygen. Time-lapse microscopy revealed that individually cultured embryos were delayed reaching the 8-cell stage (P<0.01), and the
resulting blastocysts had fewer cells and a reduced proportion of inner cell mass cells compared to embryos cultured in groups (P<0.05). Increasing the drop size from 2 µL to 20 µL further reduced cell numbers of individually cultured embryos (P<0.05), presumably due to dilution of embryo-secreted factors. In support of this, the addition of embryo-conditioned media to single embryos increased cell numbers compared to controls (P<0.001).

To determine the effect of an additional in vitro stress, embryos were cultured in atmospheric oxygen. The effect of combined single culture and atmospheric oxygen on cell numbers was more detrimental than each condition alone (P<0.001), indicating that there is a cumulative effect of these stresses. Despite the differences observed during the preimplantation stages, embryos cultured individually or in groups performed equally in outgrowth assays and no differences were observed in fetal or placental weights on day 15 of pregnancy.

When considering the relevance of these findings to clinical IVF, individual culture may reduce the numbers of transferrable blastocysts available, even if no fetal consequences are apparent. Furthermore, many clinics routinely culture embryos both individually and in atmospheric oxygen, which have cumulative detrimental effects on blastocyst development.

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**Effect of DNA methyltransferases on the reprogramming of methylation during early mouse embryo development**

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Published consent withheld

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**Effect of thyroid hormones on porcine oocyte maturation in vitro.**

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The role of the thyroid hormones thyroxine (T4) and triidothyronine (T3) in vitro embryo production has not been widely studied. In cattle the addition of T4 plus T3 to in vitro oocyte maturation media has been shown not to influence blastocyst rate or cell number (1). However it was suggested that these hormones may have beneficial effects in species where in vitro maturation is extended such as the pig and human. The aim of the present study therefore was to determine whether the addition of T4 and/or T3 to defined porcine in vitro maturation media could increase in vitro embryo production. Porcine ovaries were obtained from a local abattoir and small antral follicles 3-6mm in diameter were aspirated in the laboratory. Cumulus – oocyte complexes were matured in BOMED maturation media plus PVA containing 0, 25, 50 or 100ng/ml of T4 plus T3 in experiment one or 0, 25, 40 or 100ng/ml of T3 in experiment two for 44 hours. Following maturation COCs were coincubated with 5 x 10⁵ sperm /ml of mixed boar semen for 6 hours. After fertilisation the cumulus cells were removed and the zygotes cultured in PZM-5. The number of oocytes that developed to blastocyst stage on day 5 and 6 stage and differentially stained to determine day 6 blastocyst cell number. In the first experiment the addition of 50 ng/ml of T4 and T3 increased day 6 blastocyst inner cell mass number compared with control (P<0.05; 7.0 vs 4.0 respectively.). In experiment two the addition of 50 ng/ml of T3 increased day 5 blastocyst rate (P<0.05; 46 vs 36.5% respectively). In conclusion our results suggest that the addition of T4 and/or T3 to defined porcine maturation media have beneficial effects for in vitro embryo production.


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**Multipotent cell types in primary fibroblast cell lines used to clone pigs using somatic cell nuclear transfer.**

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We have previously demonstrated that the use of porcine mesenchymal stem cells (MSCs) isolated from the bone marrow can increase the proportion of somatic cell nuclear transfer (SCNT) embryos that develop to the blastocyst stage compared with adult fibroblasts obtained from the same animal (1). The aim of the present study was to determine if MSCs are also present in primary cultures of adult fibroblasts which are commonly used for cloning live animals. To do this we chose two primary adult cell lines that we have previously used to clone pigs. Single cells were isolated using low-density plating and then expanded. Cells were then differentiated to adipocytes, chondrocytes and osteocytes using protocols used previously by us for porcine MSCs (1). After seven days of culture, 57/90 (63%) of colonies for Line 1 displayed a typical fibroblast morphology, while the
Thiazovivin, a Rho kinase inhibitor, improves stemness maintenance of bovine embryo-derived pluripotent stem cells under chemically defined culture conditions

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Despite numerous reported attempts, successful isolation of genuine bovine embryonic stem cells has been rare. Previous studies have shown that Thiazovivin, a Rho-associated kinase inhibitor, improves the survival and self-renewal of human embryonic stem cells. The present study demonstrated the effect of Thiazovivin on the derivation of bovine embryo-derived pluripotent stem cells. The attachment rates of blastocyst and embryonic cell clumps onto feeder cells in the Thiazovivin treatment group were significantly higher than those of the control. The pluripotency markers of OCT4 and NANOG, and the adhesion molecule E-cadherin were increased by Thiazovivin treatment. This study suggests that Thiazovivin treatment improves the maintenance of stemness in a putative embryo-derived pluripotent stem cell population by promoting the expression of pluripotency marker genes as well as enhancing the expression of E-cadherin resulting in an increase in cell adhesion. This study was supported by a grant from the National Research Foundation of Korea (NRF-2006-2004042, and no. 20140504777 through the Oromaxillofacial Dysfunction Research Center for the Elderly at Seoul National University) and the Technology Development Program for Agriculture and Forestry, Ministry of Agriculture, Food and Rural Affairs (MAFRA; 111160-04), Republic of Korea

Grey Level Co-occurrence Matrix (GLCM): A novel method to access the texture of mouse embryos derived from assisted reproductive technology (ART)

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Recent insights suggest that the surrounding environment of the pre-implantation embryo is likely to alter long-term trajectory. The periconception environment therefore represents a critical window for programming fetal growth. This study evaluated the impact of the two major clinical components of assisted reproductive technology (ART), embryo culture and ovarian hyperstimulation, on embryo development by using three metabolic markers: Peroxyfluor 1 (PF1), to assess hydrogen peroxide levels, Monochlorobimane (MCB), to assess reduced glutathione abundance and Mitotracker Deep Red, to detect active mitochondria. In addition to assessment of the embryos based on morphology and staining intensity, textural analysis using Grey Level Co-occurrence Matrix (GLCM), a second-order statistical model was used to evaluate PF1, MCB and MTDR staining, providing a robust metric of embryo health. Embryos were collected 48, 60 and 88 h post-hCG treatment, corresponding to the 2-cell, 8-cell and blastocyst stage. Our results showed that embryos derived from ovarian hyperstimulation had significantly higher intensity and texture heterogeneity of active mitochondria and hydrogen peroxide as compared to the natural cycling embryos. Embryos exposed to embryo culture displayed variations in texture of active mitochondria, although there was no change in intensity. Our data provide strong evidence that the metabolic profiling and texture were modified in embryos derived from ART. To the best of our knowledge, this is one of the first studies to investigate the metabolic profiling using texture analysis in embryos, although the functional roles of each texture features are not yet well understood. This study supports that textural analysis provides a means of gaining additional information regarding sub-cellular analyses instead of using intensity measurements alone.
Hyperglycaemic stress increases blastomere heterogeneity in pre-implantation mouse embryos

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A characteristic of post-compaction embryo development is the formation of gap junctions that allow for the intercellular communication through the transport of ions, metabolites and signalling agents. However, it is not until after the 8-cell stage of development that mouse embryos develops these connections. Pre-compaction blastomere metabolic variability has been proposed as the basis of embryonic heterogeneity, and is hypothesised to be exacerbated by the absence of gap junctions. To examine blastomere heterogeneity as a consequence of cellular stress, we utilised a model of hyperglycaemic stress and analysed pathways involved in changes in metabolism and DNA damage and repair.

CBA F1 mice were stimulated with 5 IU eCG/5IU hCG, and cumulus oocyte complexes were collected 16 h post hCG and fertilised in vitro. One-cell embryos were cultured in either control (5.6mM glucose) or hyperglycaemic (30mM glucose) media for 20 hours until cleaved, before being metabolically assessed using the mitochondrial activity probe, Mitotracker Deep Red (MTDR, 200nM), a specific H2O2 fluorophore, peroxyfluor-1 (PF-1, 20μM) and a reduced GSH probe, monochlorobimane (MCB, 12.5mM). A grey-scale co-occurrence matrix (GLCM) was used for the first time on phase contrast images, to quantify its potential as an additional non-invasive method to assess embryo viability. The images were analysed for intensity and different textural features such as the degree of heterogeneity, homogeneity, smoothness and entropy using GLCM.

We found that embryos exposed to hyperglycaemic stress demonstrate a higher degree of mitochondrial activity, but not of the other metabolic measures. Phase contrast images of pre-implantation embryos revealed that hyperglycaemic embryos were more heterogeneous and were less ‘smooth’ than control embryos, supporting the hypothesis.

The reduction in melatonin level may contribute to the pathogenesis of ovarian cancer

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Introduction: Ovarian cancer is the third common gynaecological malignancy and the leading cause of death in gynaecological cancers. Studies have suggested that changes in circadian rhythms such as bright-light exposure may affect female reproductive physiology. Night shift work is associated with higher risks of breast and endometrial cancer due to lower melatonin production by the pineal gland. Other studies have suggested that the season of birth may be an important environmental risk factor for developing gynaecological cancers. Melatonin is a lipid soluble hormone whose level changes with circadian rhythm. Melatonin has multifaceted functions, including direct free radical scavenging and inhibition of cancer cell growth. However, whether there is an association between the circulating levels of melatonin and the risk of developing ovarian cancer is unclear.

Methods: Serum from women with ovarian cancer or healthy women were collected and the level of melatonin was measured. In addition, the expression of melatonin receptors (MT1 and MT2) were measured in ovarian cancer tissues by immunohistochemistry.

Results:
1. The incidence of ovarian cancer was not associated with the season of birth in women with ovarian cancer.
2. The serum levels of melatonin were significantly lower in women with ovarian cancer compared with healthy women (p<0.05). However, there was no significant difference in melatonin levels among patients who were born in spring, summer, autumn and winter.
3. Immunohistochemistry demonstrated that the expression of melatonin receptors (MT1 and MT2) was reduced in ovarian cancer tissue compared to normal ovary tissues.

Conclusion: Our results demonstrate although there is no association between the season of birth and the risk of developing ovarian cancer, the lower levels of melatonin detected in serum of women with ovarian cancer may contribute to the pathogenesis of ovarian cancer. This further supports that melatonin may be used as an adjuvant in cancer therapy.
The influence of ovarian hormones on the uterine immune response
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Infectious endometritis is a common pathology in many species, including horses, and can severely affect fertility. Efficiency of the uterine immune response is influenced by ovarian hormone levels throughout the oestrous cycle, but little is known about the molecular mechanisms underlying this phenomenon.

The objectives of this study were to characterise the changes in expression of immune genes in the equine endometrium caused by the introduction of Escherichia coli (E. coli) and to identify the transcriptional impact of ovarian hormone levels on these gene expression changes.

Thus, endometrial biopsies were collected from five horses before and at several time points after the inoculation of E. coli once in oestrus (follicle >35 mm in diameter, presence of uterine oedema) and once in dioestrus (5 days after ovulation). Absence of inflammatory signs was confirmed between treatments. Transcription in biopsies taken before and 3 hours after inoculation with bacteria was analysed using high-throughput RNA sequencing (RNA-Seq). Guided by these results, genes involved in the uterine immune response were further analysed at additional time points using quantitative polymerase chain reactions (qPCR) to quantify their expression levels until 3 days post infection.

By 3 hours after the introduction of bacteria, almost 2500 and 1500 genes were expressed at significantly higher levels compared to pre inoculation levels in oestrus and dioestrus, respectively. These included pathogen recognition receptors, particularly toll-like receptors TLR2 and 4 and NOD-like receptor NLRC5, genes for chemokines, including CCL 2, CXCL 9, 10 and 11 and those for antimicrobial peptides, including secretory leukocyte protease inhibitor, lipocalin 2, lysozyme and equine β-defensin 1. Further studies will characterize these genes at later time points post inoculation.

In-depth analysis of the uterine innate immune response will help to improve fertility in horses, but potentially also in other domestic animal species and humans.

Testicular activin A during the development of autoimmune orchitis in mice
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Experimental autoimmune epididymo-orchitis (EAEO) is a rodent model of chronic testicular inflammation that reproduces the pathology observed in some human infertility. Activin A, a dimer encoded by the inhibin βA (Inhba) gene is a pro-inflammatory, profibrotic cytokine, but also regulates spermatogenesis and steroidogenesis. The roles of activin A, inhibin and follistatin, both endogenous activin antagonists, were examined in EAEO.

EAEO was induced in adult mice by active immunization with syngeneic testicular homogenates (3 injections, 2 weeks apart) in complete Freund’s adjuvant (CFA) and Bordetella pertussis toxin. Controls received only CFA and toxin. Testes collected 25, 50 and 80 days after the first immunization were processed for histology and immunohistochemistry or frozen for qRT-PCR.

Age-matched untreated mice and controls showed no pathology, with activin A localised to Sertoli cells and interstitial cells. All immunised mice developed EAEO by 50 days, characterised by a >50% reduction in testis weight, complete loss of germ cells, immune infiltrates (macrophages and T cells) and a marked peritubular fibrotic response. These were accompanied by increased expression of inflammatory cytokines, tumour necrosis factor (TNF), macrophage chemotaxtractant protein-1 (MCP-1) and interleukin-10 (IL-10). Activin A immunostaining was not detectable in the EAEO testes, but the inhibin βA (Inhba) subunit encoding activin A and follistatin mRNA levels were similar to controls. Expression of Inhba mRNA and mRNAs encoding the activin receptors, Acvr1b and Acvr2b, were reduced. At 25 days, before observed testicular pathology, the testicular levels of activin A, TNF, MCP-1, IL-6 and IL-10 were increased (5 to 50 fold).

These data suggest that activin A acts as a pro-inflammatory agent in EAEO, but in mice with fully established EAEO, activin A levels decreased perhaps due to the testicular damage. Treatment with follistatin to attenuate activin A bioactivity early in testicular inflammation may reduce damage to spermatogenesis in patients with epididymo-orchitis.
Anti-Müllerian Hormone (AMH) has an increased rate of conversion to the active form after puberty.

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Anti-Müllerian Hormone (AMH) is a TGFβ superfamily member with multiple roles in reproductive biology. AMH induces regression of the Müllerian duct in male foetuses and has roles in testicular development. AMH negatively regulates ovarian follicular development in females. We have determined that AMH in blood consists of a precursor form (proAMH) and the receptor-competent form (AMH\(_{N,C}\)). Commercially available assays do not differentiate the two forms and most AMH measurements are an aggregate of proAMH and AMH\(_{N,C}\) (total AMH). We have developed a proAMH-specific assay to generate the first description of the relative quantities of proAMH and AMH\(_{N,C}\) in the normal population. An AMH prohormone index has been calculated from this data (API, [proAMH]/[total AMH] x 100) which represents proAMH as a percentage of total AMH. ProAMH concentrations were significantly higher in prepubertal boys (n=131) relative to men (n=80) (p = 0.000). Prepubertal girls (n=14) also had higher proAMH concentrations relative to women (n=18) (p = 0.032). The mean API of boys was approximately 2-fold higher than in men with no overlap between the ranges of each group (p = 0.000). The total AMH levels in girls and women were not significantly different but the mean API in girls was significantly greater than in women (p = 0.000). These data suggest that there is increased processing of proAMH into receptor-competent AMH\(_{N,C}\) after puberty, implying a greater proportion of AMH is active. The cleavage enzymes for AMH facilitate activation of multiple gonadal regulators, hence these findings may have wider implications for gonadal regulation during development.

Hypothalamic endoplasmic reticulum stress disrupts estradiol production, ovulation and cyclicity in a novel obese mouse model-Blobby mouse.

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Publish consent withheld

In Utero Exposure to the Insulin Sensitising Drug Metformin Reduces the Fertility of Male Offspring

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Metformin is a drug frequently used during pregnancy in the treatment of type 2 diabetes and disorders associated with insulin resistance. Most studies have focused efforts towards the effects of metformin on neural function and locomotion after birth, with few studies having investigated the consequences of in utero exposure to metformin during embryo or fetal development. Consequently a paucity of data exists aimed at understanding the effects of metformin on the reproductive function of offspring. The aim of the present study was to assess the effects of maternal metformin administration during pregnancy on the fertility of male offspring. Sperm quality analysis and immunohistochemistry were performed to measure these effects. A significant reduction of about 25% was observed in litter size from those males exposed to metformin in utero when compared to control males. We found no differences in tests size at puberty (25dpp: days post-partum) or at the adult stage (90dpp). This is contrary to our previous results which showed a decrease in fetal and neonatal tests size following in utero metformin exposure, and suggests a gradual return to normal growth after birth for the testes. Compared with controls, metformin exposed males had a reduction in seminiferous tubule diameter (141±1µm and 133±1µm, respectively;P<0.05), and germ cell number per seminiferous tubule (65±2 and 57±2, respectively;P<0.05) at 25dpp. There was a significant increase in the number of sperm head abnormalities from males exposed to metformin in utero. However, there were no differences in sperm mobility between groups. Moreover, intratesticular testosterone concentration remained unchanged, whereas in utero exposed males had lower LH concentrations in the pituitary. Exposed adult males presented with significantly more visceral adipose tissue. In conclusion we have shown that embryo/fetal metformin exposure has consequences on the fertility of male offspring, principally by affecting the quality of sperm.
Transcriptome analysis of the developing phallus after hormonal manipulation in a marsupial

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The molecular control of phallus development in males is still poorly understood, despite the fact that defects of penile development, such as hypospadias, are amongst the commonest developmental defects in newborn boys and the importance of the penis for reproductive function. In this study we investigated the molecular effects of hormonal manipulation of phallus development in the developing tammar wallaby.

We treated male tammar young with oestradiol-17β or castrated them at day 25 pp, to suppress normal penis development. We stimulated penis development in female young using androstanediol treatment from day 25 pp (1, 2). Phalloides of treated and control (untreated) young were sampled at autopsy at day 50 pp when the first macroscopic signs of sexually dimorphic phallus development are seen. Transcriptomes (pooled samples) were generated using RNA-seq. Transcripts were considered to be differentially expressed if there was more than a 2-fold difference in expression between treatments. Of the differentially expressed protein coding genes, most were classified as protein binding or catalytic enzymes. Expression of key regulators of penile development such as SHH, GLI2, β-catenin and EFNB2 were down-regulated in the male phallus after oestradiol-17β treatment and castration and up-regulated in female phallus after androstanediol treatment. Surprisingly, more than 97% of differentially expressed transcripts were predicted to be IncRNAs. Several coding gene-neighbouring long-non coding RNAs were identified. An IncRNA neighbouring MAFB was down-regulated in males by oestradiol-17β treatment and also by castration. Another IncRNA neighbouring EFNB2 was differentially expressed between male and female phallices, but there was no alteration in expression after any of the experimental treatments, suggesting that this sex difference might be independent of androgen.

In summary, both the expression pattern of key regulators and their neighbouring IncRNAs are sexually dimorphic and can be disrupted by a changing endocrine environment during phallus development.

2. Leihy, MW, Shaw, G, Wilson, JD, and Renfree, MB 2004 Penile development is initiated in the tammar wallaby pouch young during the period when 5α-androstan-3α, 17β-diol is secreted by the testes. Endocrinology 145 3346-3352.

Proteins expression in caput and corpus epididymis of Akodon cursor (Rodentia, Cricetidae)

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Publish consent withheld

A combination of growth factors is sufficient to promote testis development in the absence of Sry.

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Whether an individual develops as male or female is determined by commitment of the developing gonad to testicular or ovarian development. In mammals this decision is made when SRY is expressed in the pre-supporting cells of the bipotential gonad. SRY initiates expression of SOX9 and down-stream targets including FGF9. FGF9 promotes proliferation of the developing Sertoli cells, but is not considered sufficient to drive testis development. In this study we demonstrate that a combination of growth factors, including FGF9, Activin and TGFβ are sufficient to initiate testicular development, including the repression of the ovarian development, expression of key testis development genes, morphological reorganisation of the gonad and formation of laminin delineated testis cords. In addition, we demonstrate that facilitating β-catenin by blocking GSK opposes the testis-promoting activity of FGF9, Activin and TGFβ. Since development of the germline is strongly affected by signaling from the somatic cells including FGF9, we examined the impacts of FGF9 and FGF9, Activin and TGFβ on expression of male germline markers, pluripotency and the cell cycle regulation in germ cells, demonstrating that while FGF9 promotes male germine markers, the combination of FGF9, Activin and TGFβ maintain germine pluripotency. This study provides the first evidence that male sex-determination can be induced by a combination of growth factors in the absence of Sry. These findings have implications not only for understanding sex-determination in mammals, but also for non-mammalian species that do not have Sry.
Activin over-expression and subsequent testicular tumour development is associated with androgen deficiency and structural alterations in the reproductive tract of adult male Inha−/− mice

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Activins, members of the Transforming Growth Factor-β (TGF-β) superfamily of cytokines, are dimers of the inhibin βA- or βB-subunits. Functionally, the inhibins, which are heterodimers of one β-subunit and an α-subunit, are activin inhibitors. Within the male reproductive tract, expression of activin A is highest in the caput epididymis, but is extremely low in the vas deferens, while the converse is true for its binding protein, follistatin. This suggests that differential expression of activin A and follistatin may be involved in maintaining the regionalised structure and function of the male reproductive tract. The inhibin α-subunit gene knockout mouse (Inha−/−) lacks inhibin, and develops testicular tumours from about 4 weeks of age, leading to progressive testicular damage. These mice had very high levels of activin A and B, as well as elevated follistatin, in the serum and testes. At 8-10 weeks of age, Inha−/− epididymal weight was reduced by 50% compared with Inha+/− control mice. Sperm was absent or very low in the epididymis and vas deferens of the Inha−/− mice, and the ductal epithelium of these tissues were regressed, with increased fibrosis in the stroma around the ducts. Seminal vesicle weight and serum testosterone levels were considerably reduced, but luteinizing hormone (LH) levels were paradoxically normal. Although serum inhibin was reduced by 30% in the heterozygous Inha+/− mice, serum and testicular activins, follistatin and testosterone were not altered, and the epididymis and vas deferens appeared morphologically normal. In conclusion, deletion of the inhibin α-subunit gene leads to over-expression of both activin A and B, and androgen deficiency, although with normal LH. Regression of the epididymis and vas deferens also occurs, but it is not clear whether this is a direct result of activin overexpression, or loss of testicular function associated with tumour development, or both.

Quantification of granulosa cells in mouse ovarian follicles

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Chemotherapy often decreases antimullerian hormone levels and fertility in vivo, but effects on follicular granulosa cells are poorly characterized. Granulosa cell (GC) numbers in freshly isolated follicles can be used as baseline control values to develop less gonadotoxic chemotherapeutics. A review of 3 published reports indicated that GC/follicle numbers calculated from fixed histological sections of mouse ovaries were 21–60 (primary), 61–200 (secondary) and 201–600 (antral). We aimed to confirm these numbers using freshly isolated whole mouse follicles.

Mouse ovaries (n=5) were disaggregated with 2mg/mL collagenase IV. Isolated follicles were stained with DAPI or Calcein AM & Ethidium Homodimer-1 (‘Live-Dead’ stain) before fixation. The diameters and GC numbers of M1 (viable regular morphology) or M2 (viable irregular morphology) DAPI-stained follicles (n=215) were determined using fluorescent microscopy. Image J software and nuclear area. These data were validated by confocal microscopy, in which additional DAPI or Live-Dead stained M1 follicles from each size cohort were examined. Confidence intervals were calculated and data subjected to 1-way ANOVA with Tukey post-test.

The area of Homodimer-1 stained GC nuclei (n=60) was 15.68±3.26 μm2. Follicle diameters were 63±13μm (n=65), 120±20μm (n=87) and 196±32μm (n=63), and GC/follicle numbers were 73±33 (p=0.05), 197±67 (p<0.05) and 431±163 for high quality viable M1&M2 primary, secondary and antral follicles respectively.

We found that GC/follicle numbers were slightly higher than values obtained previously: 65-82 v 21–60 in primary, 183-211 v 61–200 in secondary and 390-472 v 201–600 GC in antral mouse follicles. This might be because follicles are not perfect spheres but have a polar orientation with irregular GC distribution. Wezel, I.L.v. and R.J. Rodgers, Morphological characterization of bovine primordial follicles and their environment in vivo. Biology of Reproduction, 1996. 55: p. 1003-1011.
The effect of Gas5 lncRNA on oocyte maturation and embryo development

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Female fertility is dependent on a number of factors, one of which is oocyte quality. The developing oocyte is highly susceptible to environmental stresses that have detrimental effects on oocyte and as embryo competence. During oocyte maturation, somatic cells (granulosa, cumulus cells) promote developmental competence of the oocyte. Recently, we identified the presence of GASS, a lncRNA, in human cumulus cells. In other systems, GASS regulates important cell survival pathways during stress conditions. In particular, progesterone and glucocorticoid receptors, which are known to be of great importance in female reproduction, are modulated by interacting with exon 12 of the GASS transcripts. However, the role of GASS in oocyte maturation and early embryo development still remains unknown. Using a PCR strategy, with cloning and sequencing, we have identified several prominent Gas5 transcript variants present in mouse granulosa cells, cumulus-oocyte complexes and pre-implantation embryos up to blastocyst stage. The abundance and differential splicing of Gas5 isoforms are modulated in COCs and embryos. Sequence analysis indicated that among the 12 exons in the full length gene, novel transcripts which include exon 12 but have exon 7 spliced out was the most common isoform. Additionally, using qPCR, we have quantitated the expression of Gas5 during the peri-ovulatory period, and found that Gas5 was significantly up-regulated at 8h post-hCG stimulation, with the majority of expression occurring in the granulosa cells. These findings are among the first to identify the importance of long non-coding RNA in reproductive outcomes and will contribute to our building knowledge on the impact of stresses on female fertility.

Effect of ovarian disaggregation on murine follicle yield and quality

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The development of fertility preservation protocols for oncology patients requires the isolation of follicles from ovarian tissue for in vitro maturation. Ovarian mechanical disaggregation using needles is time-consuming compared to disaggregation using enzymes such as collagenase IV (Col-IV), or FDA-approved purified collagenase 'Liberase'[1]. Ovarian disaggregation requires optimisation to maximise follicle yield whilst minimising damage. Follicle damage can be evaluated in a DAPI-stained follicle grading system[2] that defines M1 follicles as having viable normal morphology, and M4 as non-viable abnormal morphology. We aimed to optimise follicle harvest and test a newly available animal origin free (AOF) collagenase IV, which has the potential for TGA approval.

The ovaries from 3 month mice (n=7) were halved, weighed, and disaggregated mechanically without enzyme. Isolated follicles were stained with DAPI and CMXRos, and images were captured with an Olympus Brightfield BX50 microscope & Micro-Manager software. Follicular diameters and staining were measured using Image J. Follicle yields analysed by 1-way ANOVA, and follicle quality grades by 2-way ANOVA with Bonferroni post-test.

The ovarian weights were 6.6±2.3mg. Most of the follicles were secondary (65%)-antral (29%) -primary (6%). Follicle yields were similar for all disaggregation methods; control 13±7 follicles/ mg ovarian tissue, Col-IV 17±10, AOF590u/mL 15±11, AOF1180u/mL 13±3. For each mouse, the highest number of M1(4±1.6) and M2(4.7±1.6) follicles were obtained after Col-IV disaggregation, and the lowest M1(2.4±1.5) and M2(2.7±1.9) after mechanical disaggregation. AOF590u/mL yields were M1(3.7±2.2) , M2(3.3±2.5), M3(3.7±2.5) and M4(3±1.7) follicles/mg. Previously yields were 30-40 follicles per immature mouse ovary[2] whereas our yields from more fibrous adult ovaries were higher, ~90 follicles/ovary. Our method 'selected' for secondary follicles, and did not yield a population representative of the original tissue. Col-IV disaggregation yielded higher proportions of high quality M1&M2 follicles than the AOF preparation.


The effects of L-carnitine treatment prior to IVF on porcine oocyte maturation and post-fertilization events

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The effects of L-carnitine treatment prior to IVF on porcine oocyte maturation and post-fertilization events

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In vitro matured (IVM) porcine oocytes utilize less endogenous lipid and have poorer developmental competence than those matured in vivo. Previous studies have indicated that oocyte developmental competence may be improved by supplementing IVM medium with L-carnitine, which stimulates lipid metabolism and has antioxidant properties. The objective of this study was to determine the effects of L-carnitine on porcine oocyte maturation and post-fertilization events. Porcine Oocyte Medium was supplemented without (control) or with 12mM L-carnitine (LC) and/or 100 µM etomoxir (Etox), an inhibitor of lipid metabolism, during the final 22h of IVM. Treatment effects on cumulus-oocyte complexes (COCs) and denuded oocytes (DOs) were compared. The concentrations of ATP and glutathione (GSH) were measured at 22 and 44h of IVM in a cohort of oocytes. After IVF, presumptive zygotes were either stained to assess pronuclear (PN) formation, or cultured for 7d to assess embryo development. The levels of both ATP and GSH in DOs were about 40% lower than those in COCs (P<0.05). In both COCs and DOs, exposure to LC did not alter the ATP levels compared with the untreated controls (P>0.05), whereas exposure to LC+Etox suppressed them (P<0.05). The concentration of GSH in LC-treated oocytes was greater than that of control oocytes (8.20 vs 7.19 pmol/oocyte; P<0.05), which in turn was greater than that of LC+Etox-treated oocytes (7.19 vs 5.44 pmol/oocyte; P<0.05). Also, the PN formation rate was significantly reduced in control DOs (30%), but not in LC-treated DOs (61%), compared with the control and LC-treated COCs (87 and 90%, respectively). Blastocyst formation rates of the Etox and LC+Etox groups were significantly lower than those of the control and LC groups. The results show that the LC treatment improved the maturation of denuded oocytes through an antioxidant protective action, and not via an overall enhancement of ATP production.

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**Verification of Connexin43 in porcine oocytes during in vitro maturation**

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Many studies of the main gap junction protein, Connexin43(Cx43), have been explored in porcine oocyte research, but most of studies have been limited to investigations of cumulus-oocyte complexes(COCs). In this study, we verified Cx43 not in COCs, but in porcine oocytes during maturation, and conducted a quantitative time course analysis. The location and dynamics of Cx43 were examined by immunocytochemistry and western blotting, respectively. COCs were cultured in NCSU23 media and processed for immunocytochemistry and western blotting at 0, 14, 28, and 42h after denuding. In addition, to distinguish whether the tip shaped Cx43 signal near transzonal projections are embedded on oolemma or just exist inside of zona pellucida, we softened zona pellucida by treatment of acidic tyroid for 3 sec to completely divide zona pellucida from plasma membrane. Subsequently we proceeded immunochemistry. In result, Cx43 signal was detected on oolemmas, transzonal projections and the surface of zona pellucidae. Western blotting showed that Cx43 band density increased from 0 to 14 h, and gradually decreased thereafter. Our results clarified that Cx43 is localized in the ooplasmic membrane through zona pellucidae and its level changes over time during culture in porcine oocytes.

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**BMF regulates primordial follicle loss during adolescence**

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In mammalian ovaries, the primordial follicle pool, known as the ovarian reserve, determines female fertility and reproductive lifespan. Cell death during ovarian development in the embryo plays a critical role in determining how many primordial follicles are established within the ovary. While much attention has focussed on the apoptotic elimination of germ cells prior to and during primordial follicle assembly, the precise mechanisms that govern the steady postnatal depletion of primordial follicles after their initial endowment remain uncharacterised. In particular, there are few studies that address follicular dynamics and primordial follicle loss during adolescence. In this study we investigated the role of Bcl-2 modifying factor (BMF), a pro-apoptotic BH3-only protein belonging to the BCL-2 superfamily, in regulating primordial follicle loss in prepubertal, adolescent and adult mouse (postnatal (PN) 20-100). Primordial follicle numbers were comparable in ovaries from WT and Bmf⁻/⁻ at PN20, 30 and 40 (WT 4641.0 ± 404.7 vs Bmf⁻/⁻ 4146.8 ± 420.5 follicles/ovary, P=0.42; WT 3899.1 ± 249.3 vs Bmf⁻/⁻ 3943.6 ± 339.2 follicles/ovary, P=0.92; WT 3804.5 ± 286.9 vs Bmf⁻/⁻ 5254.0 ± 1082.7 follicles/ovary, P=0.15). However, a 50% reduction in the number of primordial follicles was observed in ovaries from WT mice between PN40 and PN50 (WT PN40 3804.5 ± 286.9 vs WT PN50 2058.8 ± 277.1 follicles/ovary, P<0.002), while a reduction in 25% of primordial follicles was observed in ovaries from Bmf⁻/⁻ mice during this same time period (Bmf⁻/⁻ PN40 5254.0 ± 1082.7 vs Bmf⁻/⁻ PN50 3316.3 ± 428.4 follicles/ovary, P<0.05).

Collectively, these data indicate that primordial follicles are lost in significant numbers during the transition to adulthood and that BMF is required for this process. The reason for the elimination for such large numbers of primordial follicles immediately prior to the establishment of adult ovarian reserve is unknown and will be the focus of future investigations.
The effect of impaired function of BMPs by passive immunization on the protein expression of BMPR1B, FSHR and LHR in the mouse ovary

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The number and quality of ovarian follicles are important in determining the longevity and integrity of female fertility. It is well known that the survival and development of these follicles including ovulation, which results in the release of viable oocytes ready for fertilization, are controlled primarily by the gonadotropins. However, there is increasing evidence which suggests that there are several other factors such as the bone morphogenetic proteins (BMPs) which co-regulate ovarian function along with gonadotropins.

In our recent studies in an attempt to shed light on the mechanism of action of BMPs, we have created an in vivo mouse model with attenuated BMP signalling using passive immunization against BMPR1B and BMP4. The aim of this study was to investigate the localization of BMP receptor 1B (BMPR1B), FSHR and LHR in the ovaries of mice treated with anti-BMPR1B, and anti-BMP4 with and without exogenous gonadotropins (eCG).

BMPR1B was expressed in all follicle stages, FSHR was detected in primary follicles onward and LHR was absent in primary follicles but appeared in later stages. Quantitative analysis based on the intensity of fluorescent signals showed that the expression of BMPR1B, FSHR and LHR significantly increased in the granulosa cells of the pre-ovulatory and secondary follicles in mice treated with anti-BMPR1B.

Mice treated with anti-BMP4 show that the expression of BMPR1B and FSHR but not LHR increased significantly in pre-ovulatory follicles only with no effects observed in any other stages. The pre-ovulatory follicles in mice treated with eCG showed increased BMPR1B and FSHR but not LHR expression.

These results together with our previous reports in sheep and mice confirm that the attenuation of BMP signalling system can be an effective approach to sustain the development of growing follicles, ovulation and consequently overall female fertility.

Expression profile of reproductive receptors during aging of the human ovarian follicle

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Reproductive ageing is linked to the depletion of ovarian primordial follicles, that causes an irreversible change to ovarian cellular function and ultimately reduces the capacity to reproduce. Our recent research has highlighted the role of bone morphogenetic protein (BMP) signalling in the regulation of the ovulation rate in sheep, and has led us to further investigate the molecular regulation of folliculogenesis by the BMPs (Regan et al. 2015). The current study aimed to profile the expression of bone morphogenetic protein receptor (BMPR1B), follicle stimulating hormone receptor (FSHR), luteinising hormone receptor (LHR), and growth hormone receptor (GHR) and also levels of apoptosis in IVF patients (101), in a range of ovarian primordial follicle depletion. An average of 8000 granulosa cells/follicle was analysed, and the follicles ranged in diameter from 4-27 mm. The granulosa cell, surface-expressed mature receptor protein density was measured by immunofluorescent labelling via flow cytometry. Ovarian reserve was measured indirectly by the antral follicle count (AFC). AFC is the number of follicles between 2-10 mm present on day 2-5 of a cycle.

A decline in granulosal BMPR1B and FSHR density occurred at the time of cyclic dominant follicle selection, and again during the terminal stage of folliculogenesis in the ‘good ovarian reserve’ IVF patients (23-30 years (y)). The older ‘poor ovarian reserve’ patients (40+ y) experienced a reversal of this pattern. The LHR density failed to be down-regulated during pre-ovulatory maturation in the 40+ y group, and GHR density was reduced with ovarian ageing. The level of apoptosis was reduced with ovarian reserve depletion.

The study’s results demonstrate the disrupting effect that age-induced depletion of the ovarian reserve has on receptor density at the two stage-specific, critical time points of dominant follicle selection and pre-ovulatory maturation. The dysregulation is potentially responsible for the reduction in oocyte quality in older patients.

A Sensitive Mass Spectrometry Method for the Simultaneous Quantification of Adenosine Nucleotides in Oocytes and Granulosa Cells

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Oocyte energy metabolism is important for its developmental capacity and the nucleotides ATP, ADP and AMP play critical roles as energy currency of biological reactions. During the early stages of oocyte maturation, cyclic AMP (cAMP) is hydrolysed to AMP which we hypothesize can be recycled through the adenosine salvage pathway to ADP and then ATP, as an alternate energy generating pathway. Significant changes in the amount of these nucleotides may also impact oocyte energy charge (EC), an index used to measure a cell’s energy status through the assessment of the energy stored in its adenylate system. To facilitate the study of oocyte adenosine nucleotide metabolism, we developed a highly selective and sensitive LC-MS/MS method for the simultaneous quantification of low nanomolar to micromolar concentrations of adenosine, ATP, ADP, AMP, and cAMP. Metabolites were separated on a porous graphic carbon column, which offered superior retention and chromatographic resolution, however rigorous conditioning protocols were required to ensure repeatability. The method was validated using αMEM culture media containing 0.3% BSA as sample matrix. The method was linear from 5nM to 10µM for all analytes with correlation coefficients above 0.998. The recovery ranged from 77% to 107% from 20nM to 5µM. Precision (%CV) was below 15% from 50nM. The limits of detection and quantification were 5nM and 10nM, respectively. The method was successfully applied to quantify nucleotides in COV434 granulosa cell conditioned media and cell extract. The mitochondrial uncoupler CCCP decreased nucleotide levels in media 2-5-fold, and cAMP modulators forskolin and IBMX increased cAMP secretion 14-fold. Nucleotide concentrations from these samples ranged between 5-90nM. Simultaneous quantification of ATP, ADP and AMP enabled calculation of the EC. Mouse oocyte EC was 0.88-0.92 throughout maturation, indicating that the EC is buffered. This novel mass spectrometry method will allow detailed interrogation of energy generating systems in oocytes.

The pan-sirtuin inhibitor, nicotinamide, disrupts the meiosis I-to-meiosis II transition in mouse oocytes

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In oocytes, exit from meiosis I (MI) is immediately followed by entry into meiosis II (MII) after which oocytes arrests at metaphase II awaiting fertilization. This unique MI-to-MII transition necessitates partial inactivation of cyclin-dependent kinase 1 (Cdk1) brought about by inhibitory Cdk1 phosphorylation and destruction of the Cdk1 co-activator, cyclin B1, mediated by the anaphase-promoting complex (APC). Following extrusion of the first polar body (PBE), which marks exit from MI, re-establishing and maintaining Cdk1 activity is important for assembling a fully formed bipolar spindle with aligned condensed chromosomes typical of metaphase II arrest. Sirtuins are NAD⁺-dependent deacetylases that are key for multiple cellular processes. Here we investigate the effect of the pan-sirtuin inhibitor, nicotinamide (NAM), on mouse oocyte maturation. Culturing oocytes in 10mM NAM during MI had no effect on rates or timing of PBE or on spindle assembly during MI. Unexpectedly however, examination of NAM treatment may be related to increased APC-mediated proteolysis as we also found increased levels of the APC co-activator, Cdc20, during MI exit. Collectively, these results indicate that NAM-induced sirtuin inhibition led to excessive Cdk1 inactivation during MI exit thereby causing oocytes to exit meiosis into an interphase-like state.

Insulin impacts cumulus oocyte complex maturation and early embryo development in vitro

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Insulin is a vital molecular component of mammalian glucose control with resting levels changing significantly in patients suffering from diabetes mellitus. As a sole genetic cause of diabetes has been ruled out and some research is leading towards
epigenetic causes; understanding the impact to the pre-implantation embryo is becoming a major component of diabetic research. Many of these studies are performed in vitro and interestingly, to date, no known studies have investigated the effect of varying levels of insulin and have only focused on varying glucose levels. The research performed here studies embryonic development using cumulus oocyte complexes derived from PMSG stimulated mice at 46 h before undergoing in vitro maturation (IVM) in varying levels of insulin (0.17pM, 1.7pM, 170pM and 1700pM). IVM is also performed at these insulin levels both with and without the presence of glucose (30mM) to mimic different stages and types of diabetes mellitus, before in vitro-fertilisation occurs. Analysing cumulus expansion using the cumulus expansion index (17h post FSH in vitro), cleavage rate at 2-cell (24 hours post fertilisation) and blastocyst rate (4 days post fertilisation) we identified that the presence of insulin significantly reduced the cleavage rate at all concentrations compared to the control without insulin (p=0.49), while only significantly reducing cumulus expansion at the lowest level of 0.17pM (p=0.038). There was no effect of either insulin or glucose on blastocyst rate; however the addition of high glucose resulted in a significantly higher cumulus (p=0.047) expansion score and a lower cleavage rate (p=0.002) supporting previous studies work. These findings suggest that considering insulin as well as glucose in epigenetic research of diabetes may be a priority and provides reasoning to further investigate the causes of the impact of insulin such as; gene expression and metabolic activity.

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Plasma Anti-Mullerian Hormone is related to oestrus expression in lactating sows

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In the pig industry, stimulating a fertile oestrus during lactation allows piglet weaning ages to be increased without impairing farrowing frequency (Soede et al., 2012). Therefore, selection of sows with a high propensity to express lactation oestrus would be beneficial. Anti-Mullerian Hormone (AMH) concentration is a reliable indicator of ovariatic reserve in many species and plasma AMH is positively related to puberty response in pigs (Reed et al., 2013). We hypothesised that lactating sows with high AMH on day 18 post-parturition will be more likely to express lactation oestrus.

On day 18 post-parturition, 54 Large White x Landrace lactating multiparous sows had a blood sample collected and commenced daily 15 minute boar contact. Sows were artificially inseminated at first detection of lactation oestrus and slaughtered 30 days post-insemination. Ovulation rate and embryo number were recorded. Plasma AMH was measured with the Human MIS/AMH DuoSet ELISA Kit (Beckman Coulter, Roissy, France) previously validated for use in bovine. Sows were divided into HIGH (39.2 ± 5.3 ng/mL) or LOW (5.4 ± 5.5 ng/mL) AMH groups using the median value of AMH. An ANOVA was used to determine the effects of HIGH or LOW AMH on all variables (GenStat Version 11, VSN International, England, UK).

More sows with HIGH AMH expressed a lactation oestrus (96% versus 67%, P<0.01). HIGH AMH sows also had a higher ovulation rate (23.7 ± 0.7 versus 21.4 ± 0.9; P<0.05); however, plasma AMH did not affect embryo survival (HIGH AMH, 62.8 ± 4.6%, versus LOW AMH, 63.3 ± 5.7%; P>0.05). Therefore, this experiment shows that plasma AMH on day 18 post-parturition is a good indicator of sows that will exhibit oestrus in lactation.


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INSR rs2059806 polymorphism and the risk of preeclampsia

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Introduction: Preeclampsia is a pregnancy specific disease that occurs in 2-8% of pregnancies and is a leading cause of maternal morbidity and mortality. Increasing evidence suggests that the effects of preeclampsia on a woman's health are not restricted to the pregnancy but that preeclampsia could represent a risk factor for later life vascular and metabolic diseases. The INSR rs2059806 single nucleotide polymorphism (SNP) is a risk factor for essential hypertension, type 2 diabetes and metabolic syndrome. We investigated the association of this polymorphism with preeclampsia.

Methods: The association of the INSR rs2059806 SNP with preeclampsia was tested in 123 Caucasian preeclamptic women and 1185 controls and replicated in an independent cohort of 175 Sinhalese preeclamptic women and 171 controls. The Caucasian women were recruited from the SCOPE study in Adelaide and Auckland and the Sinhalese women were recruited in Colombo, Sri Lanka. Preeclampsia was diagnosed using international guidelines. The controls consisted of women who had
uncomplicated pregnancies. DNA was extracted from peripheral blood collected from women and was genotyped using the Sequenom MassARRAY system. The genotype frequencies of the preeclamptic women were compared with controls using chi squared test.

Results: In the Caucasian cohort, the prevalence of the INS R rs2059806 AA genotype was increased among preeclamptic women [OR(95%CI)=3.1(1.6-5.8), p=0.003]. In the Sinhalese cohort, the prevalence of the INS R rs2059806 AA genotype was increased among preeclamptic women who delivered small for gestational infants [OR(95%CI)=2.8(1.0-7.4), p=0.03].

Conclusion: The INS R rs2059806 SNP previously associated with adult vascular and metabolic diseases is also associated with preeclampsia in two independent cohorts. These findings suggest that genetic susceptibility may be implicated in the link between preeclampsia and subsequent vascular and metabolic diseases.

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Pseudopregnancy Induction in the Spiny Mouse (Acomys cahirinus)

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Background: The spiny mouse is a precocial rodent, particularly comparable in relative gestation length, fetal development and embryo cleavage rate to that of humans. Utilisation of such a model will enable us to undertake comprehensive embryological studies; the ultimate goal to achieve improved success rates of IVF. To do so, we require a robust technique to induce pseudopregnancy. Aims: We sought to trial 3 protocols previously used in rodents in spiny mice. We hypothesised progesterone would induce decidualisation, establishing pseudopregnancy. Methods: Females aged between 90-150 days were divided randomly into one of several groups (Table 1). Spiny mice were deemed pseudopregnant if presenting with an extended luteal phase, characterised by >4 consecutive days of leukocytic smears.

Table 1: Treatment groups and vaginal smear protocols for inducing pseudopregnancy

<table>
<thead>
<tr>
<th>Group # Subjects (n)</th>
<th>Treatment</th>
<th>Smears Conducted</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>Every two days</td>
</tr>
<tr>
<td>2</td>
<td>Progesterone 4 mg</td>
<td>Daily after treatment</td>
</tr>
<tr>
<td>3</td>
<td>Progesterone 4 mg</td>
<td>Day 3 onwards after treatment</td>
</tr>
<tr>
<td>4</td>
<td>Mechanical Stimulation</td>
<td>Daily</td>
</tr>
<tr>
<td>5</td>
<td>Mechanical Stimulation</td>
<td>Day 3</td>
</tr>
<tr>
<td>6</td>
<td>Sterile Mating</td>
<td>Daily</td>
</tr>
<tr>
<td>7</td>
<td>Sterile Mating</td>
<td>Day 3</td>
</tr>
<tr>
<td>8</td>
<td>Progesterone 2 mg</td>
<td>Day 3</td>
</tr>
<tr>
<td>9</td>
<td>Progesterone 5 mg</td>
<td>Day 3</td>
</tr>
</tbody>
</table>

Results: The average length of luteal phase in untreated animals was 2.8 ± 0.2 days. This was significantly prolonged by 3-5 days in most groups, excluding 2 and 5. Though the luteal phase was prolonged in 7, 50% of subjects exhibited delayed pseudopregnancy. 40% of subjects from 8 experienced prolonged oestrus by 1-2 days. Aims: We found a single dose of progesterone (2-5 mg) was the most efficacious method of immediate pseudopregnancy induction. Altered concentrations did not have an effect on luteal phase length, hence any dosage within this range may be used. Ongoing analysis will examine hormonal profiles. Embryo transfers will be conducted to confirm protocol success.

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Predicting Pregnancy Complications from Maternal Buffy Coat DNA at 15 Weeks Gestation

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Pregnancy complications such as preeclampsia (PE), preterm birth (PTB), small for gestational age (SGA), and gestational diabetes mellitus (GDM) occur in 25% of first pregnancies and can threaten the health of mother and/or baby. Currently, no reliable biomarkers exist in clinical practice that can predict which women are likely to have a complicated pregnancy. This is particularly important in first pregnancies where there is no prior pregnancy history to inform the clinician. The SCOPE (SCreening fOr Pregnancy EndpointS) international consortium has a biobank and detailed clinical and lifestyle database for nearly 6,000 women pregnant for the first time. In Adelaide, 1169 women were recruited prospectively with detailed clinical and lifestyle information, biological samples from mother, baby and father, as well as the known outcome of the pregnancy. Of the 1169 Adelaide SCOPE women 861 had uncomplicated pregnancies, 93 developed PE, 95 delivered SGA babies, 69 delivered preterm and 51 had GDM. Since the mechanisms by which environmental factors alter gene expression are thought to be epigenetic, and the most characterised epigenetic mechanism is DNA methylation, we investigated whether differential DNA methylation identifies biomarkers that predict pregnancy complications, either alone or in combination with clinical
characteristics and genetic information. To identify biomarkers for predicting pregnancy complications, DNA was extracted from the buffy coat from maternal blood samples collected at 15 weeks gestation. Our preliminary data from 8 PE, 8 PTB and 8 uncomplicated term pregnancies suggests that analysis of DNA methylation could generate reliable diagnostic markers to predict pregnancy outcome, using a methodology known as methylation-sensitive genotyping-by-sequencing (MS-GBS) or methylation restriction enzyme sequencing (MRE-Seq). Currently we are analysing more than 400 samples from women with the 4 common pregnancy complications and uncomplicated pregnancies to validate DNA methylation changes that can be used for screening maternal blood samples for pregnancy complication prediction.

### Molecular analysis of the human placental SLC13A4 sulphate transporter: relevance to fetal growth and development.

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Nutrient sulphate is important for numerous cellular and metabolic processes, particularly in fetal growth and development. Sulphate is supplied from mother to fetus via the placenta. Previously, we localised expression of the SLC13A4 sulphate transporter to the syncytiotrophoblast layer of human (and mouse) placentae, where it is proposed to mediate transport of sulphate between mother and fetus. The consequences of perturbed SLC13A4 function on human fetal development is unknown but warrants investigation based on fetal demise in Slc13a4 null mice.

In this study, we curated 52 known genetic variants in the human SLC13A4 gene from the NCBI and NHLBI GO ESP databases and further characterized the functional consequences of six variants (L72S, F309C, V512M, I569V, N299S and E359Q) which are conserved across multiple species and predict perturbed structural stability.

EGFP-SLC13A4 fusion proteins expressed in MDCK cells showed sorting of control and missense variants (N299S, E359Q, V512M and I569V) primarily to the apical membrane, whereas SLC13A4 harbouring the F309C variant was sorted to both apical and basolateral membranes. The L72S frameshift variant was retained intracellularly with no EGFP signal detected on the plasma membrane. Functional analysis of the variants using a radiotracer ³⁵S-sulfate uptake assay, showed similar sulfate uptake between control SLC13A4 and the F309C, E359Q, V512M and I569V variants, whereas L72S completely abolished SLC13A4-mediated sulphate uptake.

This is the first study to functionally characterise known variants in the human SLC13A4 gene. Our findings show complete loss of function for L72S, and suggest that F309C leads to mis-sorting of SLC13A4. Further studies are warranted to assess the consequences of genetic variants in SLC13A4 on sulphate transport function in vivo and on fetal outcomes. These studies have the potential for development of prenatal screening for SLC13A4 mutations and genetic counselling.

### Feeding caffeine to gestational sows reduces stillbirth rates

**Brooke Dearlove¹, Karen Kind¹, Kathy Gatford³, William van Wettere¹**

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In the pork industry, the number of viable piglets born is a primary determinant of profitability. However, the incidence of stillborn piglets remains high, and is likely to increase with continuing selection for high litter size (Ke Kerr JC, Cameron ND (1995) Animal Science 60, 281-290.

Feeding caffeine to gestational sows for three days prior to parturition would reduce the number of still births.

Ninety five multiparous (parity 3.2 ± 0.2), Large White / Landrace sows were housed in farrowing crates from 5 days prior to their due date, sows were fed a capsule containing either 2 g (Caffeine group) or three times per day at feeding or an empty capsule (Control) three times per day at feeding. Treatments continued until commencement of farrowing. The number of liveborn, stillborn and mummified piglets was recorded at farrowing. Results were analysed using a univariate general linear model (IBM SPSS Statistics 21) with birth order, treatment, parity, pen and room as fixed effects and litter size as a covariate.

Gestation length was increased in sows treated with caffeine (Caffeine: 116.6 ± 0.3 days versus Control: 115.5 ± 0.3 days; P <0.05). Total litter size did not differ between treatment groups. Caffeine treated sows had more live born piglets (Caffeine: 11.65 ± 0.22 versus Control: 11.01 ± 0.23; P <0.05) and fewer still born compared to controls (0.29 ± 0.09 versus 0.67 ± 0.15; P <0.05).

This study has demonstrated that oral caffeine administration can influence parturition outcomes in sows. Further studies are underway to determine the effects of caffeine on piglet viability and the underlying mechanisms.

Exploring the potential of mesenchymal stem cell transplantation into human placentae in vitro
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Background: In the placenta, mesenchymal stem/stromal cells (MSC) are located in a perivascular niche and exhibit a tendency to differentiate into endothelial cells, suggesting a role in vascular development. Animal studies have shown that transplanted MSC stimulate angiogenesis in several different tissues. Thus, placental MSC may be therapeutically useful in pregnancy disorders such as intrauterine growth restriction where placental vascularisation is insufficient. This study aimed to assess the behaviour of transplanted MSC in human placental explants in vitro.

Methods: MSC isolated from first trimester and term placentae were characterised by flow cytometry. MSC were labelled with CMRA and injected into first-trimester or term placental villous explants, or between placental villi. MSC viability post-transplantation was assessed by counterstaining explants with CMFDA after 48 or 96 hrs in culture. Fixed explants were vibratome sectioned and imaged by confocal microscopy.

Results: Following transplant, MSC migrated from the injection site to the villus tips and in some instances surrounded placental vessels. 89.1% (+3.7% SE, n=9 explants, 3 placentae) of first-trimester MSC injected into first-trimester explants were viable after 48 hrs of culture. Term MSC injected into blood vessels were only viable after 48 hrs if they migrated out of the vessels into the villus stroma (51.8% ±6.9% SE, n=9 explants, 3 placentae). After 96 hrs, no viable MSC were evident within term or first-trimester explants, nor was the explant tissue itself viable. When transplanted between placental villi, MSC formed cellular aggregates from which some MSC appeared to cross into the villus stroma.

Conclusions: MSC can be successfully transplanted into placental villi, and MSC transplanted into the intervillosus space can cross into the placenta. However, MSC viability in this model is limited by the overall viability of the explants, highlighting the need to use an in vivo model to study MSC behaviour over a longer timeframe.

RNA interference-mediated knockdown of BMP receptors in cultured bovine theca cells: effects on androgen secretion and cell proliferation/survival
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Introduction
BMPs and other TGFβ family members are firmly implicated as intraovarian regulators of ovarian follicle development and steroidogenesis. Whilst multiple TGFβ family ligands and receptor subtypes are known to be expressed in ovarian theca (TC) and granulosa cells, information on which ligand-receptor interactions are important for particular physiological actions is limited. Here we used a primary bovine theca cell culture model to examine whether RNAi-mediated knock down of individual BMP receptors affects androstenedione (A4) secretion and cell proliferation/survival.

Methods
TC from 4-6mm follicles were cultured for 7 days in serum-free medium with LH (150 pg/ml) present from day 3-7. On days 4 and 5 cells were exposed to RNAi duplexes (100nM) targeting bovine BMPR1A (ALK3), BMPR1B (ALK6) or BMPR2; controls included cells treated with non-silencing control RNAi, transfection reagent (TR) alone or no treatment. A4 secretion during day 6-7 was measured by ELISA and viable cell number was determined by neutral red assay at the end of culture. RNA extracts were harvested from representative wells for examination of target gene knockdown using RT-qPCR (β-actin normalisation control). Results are based on 4 independent batches of cells.

Results and Discussion
RT-qPCR indicated ~85% knockdown of BMPR1A and BMPR1B and ~75% knockdown of BMPR2 mRNA expression by the relevant RNAi; non-silencing controls and TR-only controls had similar expression levels to untreated control cells. A4 secretion was raised (P<0.01) by 3.6, 2.6 and 3.2-fold in cells treated with BMPR1A, BMPR1B and BMPR2 RNAi respectively while corresponding cell number was reduced (P<0.05) by 40, 18 and 35%. The results indicate that endogenous TC-derived TGFβ-family ligands that signal via BMPR1A, BMPR1B and/or BMPR2 exert autocrine/paracrine role to suppress thecal androgen production and enhance cell proliferation and/or survival. Further experiments will examine the extent to which knockdown of individual receptors affects responsiveness to exogenous BMP ligands.

Association between growth rates, age at first calving and subsequent fertility in Holstein heifers
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Publish consent withheld
Maternal Obesity negatively impacts on fetal kidney development, maternal health and birth outcomes in an Indigenous Australian cohort

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Background: Chronic disease in indigenous populations around the globe is prolific. There is a worldwide epidemic of obesity, a leading cause of chronic disease. The impact of obesity on pregnancy outcome is poorly understood as are the effects of maternal obesity on fetal organ development.

Methods: We studied a cohort of Indigenous pregnant women. Maternal height, weight, BMI, and %body fat were measured as well as fetal size and fetal kidney volumes; the latter using ultrasound. Maternal health and birth outcomes were recorded.

Results: The median maternal weight and BMI of the cohort was 85.34kg (range: 45-148kg) and 30.74kg/m² (15-52 kg/m²), and %body fat was 43.65% (17-63%). Maternal BMI was positively associated with birth weight (rho=0.32; p=0.005) but not with length of gestation. Both maternal BMI and %body fat were negatively associated with the infant’s combined kidney volume/ fetal weight (rho=-0.357, p=0.016 and rho=-0.406, p=0.014). 6.2% of the cohort developed gestational diabetes (GDM) and delivered earlier (p=0.002). These babies had a median birthweight centile that was significantly greater than that of babies whose mothers did not have GDM (p=0.031). GDM women had higher urinary protein/creatinine and albumin/creatinine (p=0.047 and p=0.024). There was no effect of maternal GDM on fetal kidney size.

Kidney volume is a surrogate measure of nephron number. The inverse correlation between kidney size and measures of maternal obesity implies that these babies are at risk of developing chronic renal disease. The mechanisms responsible for this association between kidney size and maternal obesity are unknown but the data highlight the need to reduce obesity in this population.

Sex differences in the human placental methylome

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In utero, females limit their growth, thereby maintaining a greater placental reserve capacity. Males on the other hand, extract maximal nutrients from their placenta which likely underpins the consistent observation that males are born heavier than females. The mechanisms that give rise to these sex differences are unknown. Previous work in our laboratory has revealed sex biased gene expression in the term placenta from uncomplicated pregnancies in an integrative meta-analysis. It was found that > 140 genes were differentially expressed between male and female placentas; > 60 % of these genes were autosomal. A possible mechanism for these sex differences is DNA methylation, however it is unknown if autosomal DNA methylation patterns differ between male and female placentas. We hypothesised that the sex differences in gene expression evident in the term placenta are the result of altered DNA methylation patterns.

To test our hypothesis, we undertook a bioinformatic approach by combining six publically available DNA methylation microarray datasets, featuring term placental tissue from 45 uncomplicated pregnancies (42 % male, 58 % female), thereby maximising statistical power. All F-values were corrected for false discovery by calculating the family wide error rate (FWER). We identified > 160 differentially methylated regions (DMRs) when comparing male and female placentas (p < 0.05, FWER < 0.01). The DMRs were all situated on the sex chromosomes with > 80 % from the X chromosome. We then mapped these DMRs to the genome, to determine if they related to placental gene expression. It was found in many cases that DNA methylation was correlated with gender expression. For example XIST was found to be hypermethylated in males compared to females, but is expressed more highly in females. These results indicate that DNA methylation may be an underlying mechanism for some of the sex differences in placental gene expression.
Identification of salivary and placental proteins associated with subsequent allergic disease in childhood

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3. Adelaide Proteomics Centre, School of Biological Sciences, The University of Adelaide, Adelaide, South Australia, Australia

Allergic disease has risen to epidemic proportions during recent years. It has become evident that prenatal events play a critical role in determining disease susceptibility via environmental influences on placental function and fetal programming. We hypothesize that childhood susceptibility to allergy is increased through significant alterations in placental gene expression and products of identified genes are altered in the saliva of allergic children. We aim to identify the proteins associated with childhood allergy using placental tissue from two populations of women whose children have different risks of allergic disease susceptibility. Then, we will determine whether these altered genes are detectable in the children’s salivary proteins. The objective of the study are to identify salivary proteins that could be a potential biomarker, to identify allergy risk in newborns and to identify targets proteins for early allergy interventions. Placenta and saliva will be examined using a proteomic approach that involves quantitative label-free comparative MS. Saliva and placental tissue from children with no allergy will be compared to children with either asthma, eczema and rhinitis (n=18). Six candidate proteins were identified in saliva samples associated with subsequent allergic disease in childhood and will be validated. Five proteins were identified present in all the allergic phenotypes that include Human Mucin-5B and Human Mucin 5AC with the ratio of >2 and Human Serum Albumin, Human Serotransferrin and Human Triosephosphate Isomerase with the ratio <0.5 fold change relative to non-allergic samples. Moreover, one protein was present in high expression in all 3 allergic phenotypes and had very low expression with no calculated ratio when compared with control group that is Human Pyruvate kinase PKM. The current findings suggest protein expression can be altered in utero in children who subsequently develop an allergy and the altered expressions of these proteins are detectable in saliva in early life.

Plasma Anti-Mullerian Hormone and in vitro embryo quality in lactating sows

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Stimulating a fertile oestrus during lactation in pigs allows increased piglet weaning age without decreasing farrowing frequency. However it is important that ovarian follicle growth and oocyte quality is not reduced when mating in lactation. Anti-Mullerian Hormone (AMH) is a marker for ovarian reserve in many species and low AMH levels have been linked to poor oocyte quality in humans (Lekamge et al., 2007). We hypothesised that lactating sows with HIGH AMH on day 21 post-parturition would have a larger and more mature ovarian follicle pool and oocytes with greater developmental competence in vitro than sows with LOW AMH.

On day 18 post-parturition, 33 Large White x Landrace lactating multiparous sows commenced daily 15 minute boar contact. On day 21 post-parturition, a blood sample was collected, sows were slaughtered, surface ovarian follicles were counted and cumulus-oocyte complexes were collected for in vitro embryo production (Kelly et al., 2010). Plasma AMH was measured with the Human MIS/AMH DuoSet ELISA Kit (Beckman Coulter, Roissy, France) previously validated for bovine. Sows were divided into HIGH (77.0 ± 8.5 ng/mL) or LOW (9.0 ± 9.0 ng/mL) AMH groups using the median value of AMH. ANOVA was used to determine the effects of HIGH or LOW AMH on all variables (GenStat Version 11, VSN International, England, UK).

Sows with HIGH AMH tended to have more surface follicles > 6 mm (17.8 ± 2.1 versus 12.4 ± 2.1; P<0.1). AMH concentration did not affect embryo cleavage or blastocyst development rates, however, blastocysts from HIGH AMH sows had greater total cell number than those from LOW AMH sows (37.2±3.6 versus 21.6±4.3; P<0.05). These results indicate that HIGH AMH sows have a more mature ovarian follicle pool during lactation, and while embryo development rates were unaffected by AMH levels, embryo quality was improved in HIGH AMH sows.

Chromatin Pattern and Status of Global DNA Methylation in Human Spermatozoa
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Chromatin compaction and methylation status are biomarkers to detect the pattern and quality of sperm DNA prior to ART. The purpose of this study is to compare two criteria of sperm chromatin compaction and global methylation status in relation to the functional quality of human spermatozoa. The confocal microscopy and flowcytometry showed the Immunocytofluorescent pattern of ChromomycinA3 (CMA3) staining and the 5-methyl cytosine, sequencely. The CMA3positivity level showed a quality relation dependency (p<0.0001), also significant correlation (R=0.05) with the flowcytometry level of global methylation. Moreover confocal microscopy from CMA3 stained head of sperm demonstrated the spatial pattern of chromatids. Overall the results of this study support the concept that perfect spermatozoa collected from the high density Percoll fraction possesses higher compaction related to hypomethylated nuclear DNA. Interestingly the results of ChromomycinA3 assay demonstrated spatial geometry of chromatids in the sperm head. Also direct significant correlation with methylation status suggesting that during the development of spermatozoa, failure to chromatin compaction is associated with more extensive methylation of sperm DNA in poor quality of spermatozoa.

ALDH2 protects stallion spermatozoa from lipid peroxidation-induced loss of motility
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Stallion sperm membranes contain high levels of polyunsaturated fatty acids, making them particularly susceptible to lipid peroxidation. While spermatozoa of other species lose motility following peroxidative damage, stallion spermatozoa have evolved defences against this motility loss despite accumulating high levels of peroxidative adducts such as 4-hydroxynonenal (4-HNE). As stallion spermatozoa are highly dependent on oxidative phosphorylation for ATP production, this adaptation may have developed as a protective measure against elevated ROS production due to mitochondrial superoxide leakage. Subsequently, positive correlations between 4-HNE (measured flow-cytometrically using an anti-4-HNE antibody) and computer-assisted sperm assessment parameters of total motility (R=0.46), rapid motility (R=0.51), VAP (R=0.62) and VCL (R=0.55) were apparent after 48h at RT. It was hypothesised that this paradoxical relationship may be due to stallion spermatozoa possessing high levels of mitochondrial aldehyde dehydrogenase (ALDH2), an enzyme responsible for the scavenging of toxic aldehyde products, primarily 4-HNE. By virtue of its locality, this enzyme may actively remove peroxidative adducts from proteins of the sperm tail, preventing the immediate loss of motility which is observed in glycolytic spermatozoa of the human under the same conditions. PCR analysis confirmed ALDH2 expression by stallion spermatozoa, and flow-cytometric measurement of ALDH activity using the Aldefluor™ probe uncovered highly significant positive correlations between ALDH expression and progressive motility (R=0.62), rapid motility (R=0.63), linearity (R=0.41), VAP (R=0.50), VSL (R=0.55) and VCL (R=0.44). Immunocytochemistry was performed to ascertain both the locality of ALDH expression and the pattern of 4-HNE adduction in both untreated and 4-HNE treated spermatozoa. As predicted, ALDH2 was most highly expressed in the mid-piece, and 4-HNE mid-piece adducts were minimal, with adduction being limited to the post-acrosomal region and principle piece, regardless of treatment. These results indicate that ALDH2 activity is the primary mechanism for the amelioration of ROS-induced peroxidative damage and motility loss in stallion spermatozoa.

The Role of ESRP1 During Gametogenesis
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Alternative splicing plays critical roles in controlling developmental programs. To date, there is evidence that many genes splice differently during gametogenesis. The regulation of alternative splicing occurs through a network of highly combinatorial molecular interactions. Numerous RNA binding proteins (RBPs) and transcription factors are involved in this process. Esrp1 (Epithelial Splicing Regulatory Protein 1) is a cell- type specific regulator. In the literature to date there have been no investigations into the expression and function of this gene during gametogenesis. As alternative splicing is a frequent event in the ovary and testis, we initiated studies to determine whether Esrp1 has a role in spermatogenesis and oogenesis. In the current study, we examined Esrp1 gene expression in mouse in developing germ cells and somatic cells. Esrp1 was expressed in germ cells but not somatic cells. Comparison of different developmental stages of spermatogenesis (gonocytes, spermatogonia, pachytene spermatocytes and round spermatids) using droplet digital PCR showed that ESRP1 is most highly
expressed in spermatogonia. Consistent with this, immunofluorescence experiments to determine Esrp1 expression pattern of in adult testis showed distinct staining in spermatogonia. This distinct expression pattern for Esrp1 strongly suggests a specific role for Esrp1 in promoting splicing events during spermatogonial development.

Sperm motility of frozen-semen derived from epididymis in young bull and blastocyst development after in vitro fertilization in Hanwoo

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².In the present study, we examined sperm quality derived from epididymis in Hanwoo bull at 13 months (before puberty). Collected semen from epididymis of two bulls were cryopreserved in LN2. After thawing of frozen semen was examined sperm motility and sperm motility parameters by Computer Assessment of Sperm Analysis (CASA) system. Progressive motility of frozen-thawed sperm in epididymis was lower than that of before freezing. Curvilinear velocity, straight-line velocity, average path velocity and linearity of frozen-thawed sperm in epididymis were lower than those of before freezing. In addition, blastocyst development of oocytes fertilized with frozen-thawed sperm from epididymis of bulls were examined. Blastocyst development rates after fertilization in vitro with frozen-thawed sperm between epididymis and commercial semen were similar (38.5 vs. 32.0, respectively). In conclusion, sperm derived from epididymis in pre-puberty of Hanwoo bulls have low motility and many are not yet mature; however, it has fertilizing ability and blastocyst development after fertilization.

Tob1 protein is a novel regulator of gonadal function

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Mammalian gametogenesis relies on a complex program of mitotic, meiotic and differentiation processes that are strictly regulated by stage- and germ-cell specific gene expression. Tob1 is a member of the BTG/TOB family of proteins with established roles as negative regulators of cell proliferation. In mouse and human Tob1 is expressed in multiple adult tissues including the testis and ovary but the specific cell types that express Tob1 in gonads was unknown. In this study we examined murine Tob1 gene expression by droplet digital PCR in developing germ cells and sorted male germ cells (gonocytes, spermatogonia, pachytene spermatocytes and round spermatids), and in situ hybridization in adult ovary and testis. Tob1 protein expression in adult ovary and testis was done by immunofluorescence. Tob1 expression was uniformly low in developing male germ cells but increased 10-fold in developing female germ cells undergoing entry into meiosis (E15.5) compared to E12.5 germ cells. In adult testis Tob1 mRNA was most highly expressed in round spermatids. Round spermatids and oocyte in all stages of folliculogenesis were positive for Tob1 protein. Notably, a marker for P-Dcp2 in both cell types, suggests Tob1 protein may play a role in post-transcriptional mechanisms during gametogenesis.

Effect of low oxygen on the pro-angiogenic pathways of the renin angiotensin system (RAS) in a human trophoblast cell line

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Background: During the first trimester of pregnancy, normal placental development occurs in a low oxygen environment. A low oxygen environment can stimulate angiogenesis via upregulation of vascular endothelial growth factor (VEGF). High levels of expression of genes that control the activity of the placental renin-angiotensin system (RAS) occur in early pregnancy. While the RAS and oxygen can both stimulate angiogenesis, how they interact within the placenta is unknown. We postulated that low oxygen increases expression of the pro-angiogenic RAS pathway and this is associated with increased VEGF in a first trimester human trophoblast cell line (HTR-8/SVneo).
Method: HTR-8/SVneo cells were cultured in one of three oxygentensions (1%, 5% and 20%). RAS and VEGF mRNA expression were determined by qPCR. Prorenin, angiotensin converting enzyme (ACE) and VEGF protein levels in the supernatant as well as prorenin and ACE in cell lysates were measured using ELISAs.

Results: Low oxygen significantly increased the expression of both angiotensin II type 1 receptor (AGTR1) and VEGF (both \( P<0.05 \)). There was a positive correlation between AGTR1 and VEGF expression at low oxygen \( (r=0.64, P<0.005) \). Corresponding increases in VEGF protein were observed with low oxygen \( (P<0.05) \). Despite no change in ACE1 expression, ACE levels in the supernatant increased with low oxygen (1 and 5%, \( P<0.05 \)). Expression of other RAS components did not change.

Conclusions: Low oxygen increased AGTR1 and VEGF expression as well as ACE and VEGF protein levels suggesting that it activates the pro-angiogenic RAS pathway. This highlights a potential role for the placental RAS in mediating the pro-angiogenic effects of low oxygen in placental development.

### An altered proliferative phase uterine microenvironment in idiopathic infertile women

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Endometrial gland secretions are essential for successful embryo implantation. Gland development occurs during the proliferative phase and lays the foundation for the later receptive phase. Despite its importance little is known regarding the impact of endometrial gland regeneration in determining female fertility or infertility. We hypothesised that gland formation during the proliferative phase is altered in infertile women. Our aim was to compare the cytokine profile and gland density during the proliferative phase of fertile and infertile women.

Area of the glandular epithelium (GE) as a percentage of total area was determined in endometrial tissue sections collected from fertile \((n=19)\) and infertile \((n=14)\) women. The expression of 41 cytokines in proliferative phase uterine fluid of fertile \((n=15)\) and infertile \((n=15)\) women was measured using Luminox immunostaining. The samples were further grouped according to age: fertile <35 years \((n=5)\), fertile ≥35 years \((n=10)\), infertile <35 years \((n=7)\) and infertile ≥35 years \((n=8)\). Cellular localisation of transforming growth factor alpha (TGFα) and interferon gamma (IFNγ) within the proliferative phase endometrium; fertile \((n=15)\) and infertile \((n=11)\) was examined using immunohistochemistry.

There was no significant difference in GE area of infertile women compared to fertile. Interleukin-1 alpha (IL-1α) was significantly increased \((p=0.034)\) in infertile compared to fertile women. Significant elevation of CCL11 \((p=0.048)\), TGFα \((p=0.049)\), IFNγ \((p=0.033)\) and IL-1α \((p=0.047)\) was evident in infertile women <35 years compared to fertile. There were no significant differences in the ≥35 years’ group. TGFα and IFNγ localised predominantly to the GE of both fertile and infertile proliferative phase endometrium.

Our data found altered proliferative phase expression of four cytokines, most notably among women <35 years with idiopathic infertility. However, gland area was unaltered suggesting that gland functionality rather than number may underlie infertility in women, with such a failure evident during the proliferative phase.

### Investigation of the production and signalling of insulin in the endometrium

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Insulin signalling is mediated by a complex, highly integrated network that controls several processes including glucose homeostasis, protein synthesis and cell growth. Traditional paradigms hold that insulin production is restricted to the beta cells of the pancreas. However, this long held view of insulin genesis has been challenged by recent studies showing that insulin can be produced by alternative, non-pancreatic cells. For instance, insulin has been detected in porcine spermatozoa and been shown to be released from ejaculated human spermatozoa in response to glucose. Furthermore, insulin has been shown to be beneficial to spermatozoa, with the capacity to act as a pro-survival factor, improve their motility characteristics, and enhance their ability to complete an acrosome reaction. This study addresses the possibility that insulin might also be secreted by the endometrium in order to sustain spermatozoa on their extensive journey from the site of insemination to the ampullae of the Fallopian tubes where fertilization takes place. Our preliminary experiments using nested PCR, indicated that insulin mRNA was indeed present in the murine uterus. We have since confirmed these data using immunocytochemistry targeting the C-peptide, a pro-domain that is cleaved from the mature insulin protein and thus provides evidence of the active synthesis of this hormone within the uterus. Specifically, C-peptide appeared to be restricted to the endometrial epithelial cells, but underwent pronounced changes in expression levels throughout the oestrous cycle; being highly expressed at pro-oestrus and oestrus, before decreasing during metaoestrus and becoming undetectable at dioestrus. The identification of insulin production in the uterus has a wide range of implications for fertility and reproduction as well as for diabetes and obesity. Our future studies will endeavor to characterise the function of insulin produced within this environment with a particular focus on its ability to influence spermatozoa.
VAMP2 and Syntaxin 3 coordinate vesicle machinery in uterine epithelial cells during early pregnancy in the rat.  
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Uterine epithelial cells undergo extensive morphological and molecular remodelling to prepare for implantation; these changes are collectively termed ‘the plasma membrane transformation’. These changes are likely mediated by vesicular trafficking and indeed there is a large increase in the number of apical vesicles as well as an increase in vesicular activity at the time of receptivity.

This study examined the role of VAMP2 and Syntaxin 3 in the uterus during early pregnancy. Vesicle associated membrane protein 2 (VAMP2) is known to travel in vesicle membranes that constitutively fuse with the plasma membrane. Syntaxin 3 is a crucial protein involved in the delivery of proteins from the trans-golgi network to the apical surface of polarized epithelia.

Uterine tissues were collected from pregnant rats during early pregnancy for immunofluorescence and uterine epithelial cells were isolated for western blot analysis.

Immunofluorescence microscopy at the time of fertilisation (non-receptive) has demonstrated that VAMP2 and Syntaxin 3 are diffusely distributed throughout the cytoplasm of uterine epithelial cells. At the initial stage of implantation (apposition), VAMP2 remains diffused throughout the cytoplasm with granular staining in the perinuclear region. During adhesion, VAMP2 becomes restricted to the cytoplasm region above the nucleus but below the localisation of Syntaxin 3, which is found immediately below the apical plasma membrane. Western blot analysis of isolated uterine epithelial cells reveals an overall increase in the amount of VAMP2 and Syntaxin 3 from the non-receptive phase to the time of implantation.

This increase in VAMP2 and Syntaxin 3 as well as the more confined localisation at the apical cytoplasmic region of uterine epithelial cells suggests that these proteins are involved in vesicle regulation. This may play a role in maintaining directional vesicle traffic to the apical plasma membrane at the time of uterine receptivity.

Identification of Haemoglobin and possible role in pre-implantation embryo development  
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Haemoglobin is a well-described gas transport protein commonly found in erythrocytes, however, non-erythroid tissues, such as cancer cells, also express haemoglobin mRNA and protein. We previously published that granulosa and cumulus cells from murine ovarian antral follicles express haemoglobin mRNA and protein, which are hormonally regulated over the peri-ovulatory period. In this study, we investigated the gene expression of haemoglobin subunits and mediators of oxygen-carrying capacity in the early embryo, comparing in vivo to in vitro development.

Pre-pubertal CBAF1 female mice were treated with 5IU eCG/5IU hCG and mated. Embryos were collected 44, 54, 86 and 92 h after hCG treatment, corresponding to the 2-cell, 4-cell, morula and blastocyst stage. For in vitro experiments, cumulus-oocyte complexes were collected 16 h after hCG, and in vitro fertilisation and embryo culture carried out. Embryos were collected 20, 42, 55, 92 h post-in vitro culture, corresponding to the same stages.

RT-PCR revealed, for the first time, high expression of Hba-a1 and Hbb at the 2-cell stage in vivo compared to in vitro expression, which increased at the 4-cell stage, and declined to near undetectable levels by the morula stage; suggesting Hba-a1 may be switched on at the 4-cell stage and degraded at the morula stage. Haptoglobin (Hp) and 2,3-bisphosphoglycerate mutase (Bpgm) were virtually undetectable in vivo and in vitro. The function of haemoglobin within in vivo embryos remains unknown, but we propose that sequestering gases, particularly oxygen, could allow the embryo to survive in the low oxygen environment of the female reproductive tract.

Quantitative assessment of uterine receptivity prior to embryo transfer increases implantation rate to >95%  
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Embryo transfer is a commonly performed surgical technique with applications in transgenic animal production, species derivation, assisted reproduction, and scientific research. In mice, protocols typically specify pairing recipient females with vasectomised males to induce a receptive uterine environment for embryo implantation. However, this induced receptive state, termed ‘pseudopregnancy’, is not always maintained until implantation occurs. We therefore evaluated the use of a well-
characterised correlation between estrous state and exfoliative vaginal cytology to assess uterine receptivity immediately prior to embryo transfer. Eight to twelve week old virgin female CD1 mice (n=22) were paired overnight with vasectomised males. Successful mating was indicated by the presence of a vaginal plug the following morning. These dams underwent embryo transfer 3 days later with embryos obtained from superovulated four week old F1(C57BL/6 X CBA) females. Non-invasive vaginal lavage was conducted immediately prior to transfer. Dams were killed 6 days after transfer and the uterus collected for histological analysis. Embryo implantation rate in mice was 96% when quantitative cytological analysis of the lavage samples signified diestrus (n=6), whereas the implantation rate was <15% (n=16) when cytology signified other stages of estrous. This simple, quick, non-invasive measure of receptivity was found to be accurate and easily adopted, avoiding unnecessary surgery and subsequent culling of non-suitable recipients, while maximising the implantation potential of each recipient female.

Parvin during implantation and subsequent culling of non-suitable recipients, while maximising the implantation potential of each recipient female.

Proteomic and functional characterization of human endometrial epithelial exosomes reveal cargo proteins essential for embryo-maternal interactions

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Establishment of a successful pregnancy requires synergistic communication between the endometrium and the blastocyst during the pre-implantation phase. Endometrial exosomes released into the uterine microenvironment are proposed to play essential role in the implantation process. In this study, we investigated the proteomic profiles of endometrial exosomes across the menstrual cycle and examined the effects of exosomes on trophoblast function. Mass spectrometry was used to study the highly purified exosomes isolated from ECC1 endometrial epithelial cells, treated with estrogen and progesterone. From a total of 1073 exosomal proteins identified, 684 were found common, while 258 and 131 proteins were uniquely enriched in response to estrogen and progesterone respectively. Functionally, 24-hour live cell imaging showed a progressive accumulation of exosomes in HTR8 trophoblast cells, resulting an increase in adhesion response of 24% (p < 0.001). Western blot analyses of endometrial exosomes and HTR8 cell co-culture suggested that focal adhesion kinase signaling pathway may be involved in adhesion response of trophoblast cells. This study is the first to demonstrate that cargo proteins packaged within endometrial exosomes are important during pre-implantation and may offer new avenues to improve receptivity and pregnancy outcomes.

Suggested Role for Alpha-Parvin and MAPK/Erk Phosphorylation in Focal Adhesion Disassembly During Early Pregnancy in the Rat

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Alpha-Parvin is a focal-adhesion associated protein found in most cell types and our work hopes to use the protein as novel model for studying quantifiable differences in a cells ability to metastasize. Alpha-Parvin has not been looked at in the uterus before. The phosphorylation of alpha-parvin has been shown to be associated with cell movement and focal adhesion disassembly. MAPK/Erk is a protein which has been shown to phosphorylate alpha-parvin which increases cell movement and adhesive degradation. By studying the localisation of these two proteins during early pregnancy in the rat, we have been able to research the role that the protein plays in focal adhesion disassembly, as during the time of implantation these adhesive complexes in the epithelium lining the lumen disassemble to facilitate blastocyst attachment.

By determining the localization and amount of alpha-parvin and MAPK/Erk in early pregnancy, we suggest a relationship between the two in coordinating focal adhesion disassembly.

Using immunohistochemical and western blotting techniques, we looked at the amount and localisation of Alpha-Parvin during early pregnancy. Alpha-Parvin is present and basally located at the time of fertilization, which shows its association as a focal adhesion protein. At the time of implantation, when the complexes are disassembled, Alpha-Parvin is significantly decreased. We also showed that phosphorylated Alpha-Parvin has a reciprocal relationship, in that it is significantly increased at implantation suggesting its role in focal adhesion disassembly. Preliminary MAPK/Erk results also show a similar localisation to phosphorylated alpha-parvin at the time of implantation.

We show for the first time that Alpha-Parvin is phosphorylated prior to focal adhesion disassembly during early pregnancy and that this phosphorylation could be indicative of a phosphorylation-dependent focal adhesion disassembly.
Models of estrogen insufficiency have revealed new and unexpected roles for estrogens in both males and females. These models include natural mutations in the aromatase gene, as well as mouse KOs of aromatase and the estrogen receptors. Some of these roles apply equally to males and females and do not relate to reproduction, for example the bone, vascular and “Metabolic Syndrome” phenotypes. We have studied the phenotypes of several men with natural inactivating mutations in the aromatase gene as well as mice in which the gene has been disabled (ArKO mice). Some of the phenotypes of these mice are summarized below:

Infertility and lack of sexual behavior in both males and females.
Progressive defects in folliculogenesis and spermatogenesis.
Elevated gonadotropins and T levels.
Loss of bone mass in both females and males.
Metabolic syndrome with insulin resistance, truncal obesity, male-specific hepatic steatosis, and defective vascular endothelial and smooth muscle function.
Development of male-specific Obsessive Compulsive Disorder.
Many of these phenotypes are also present in aromatase – deficient humans, as will be presented.