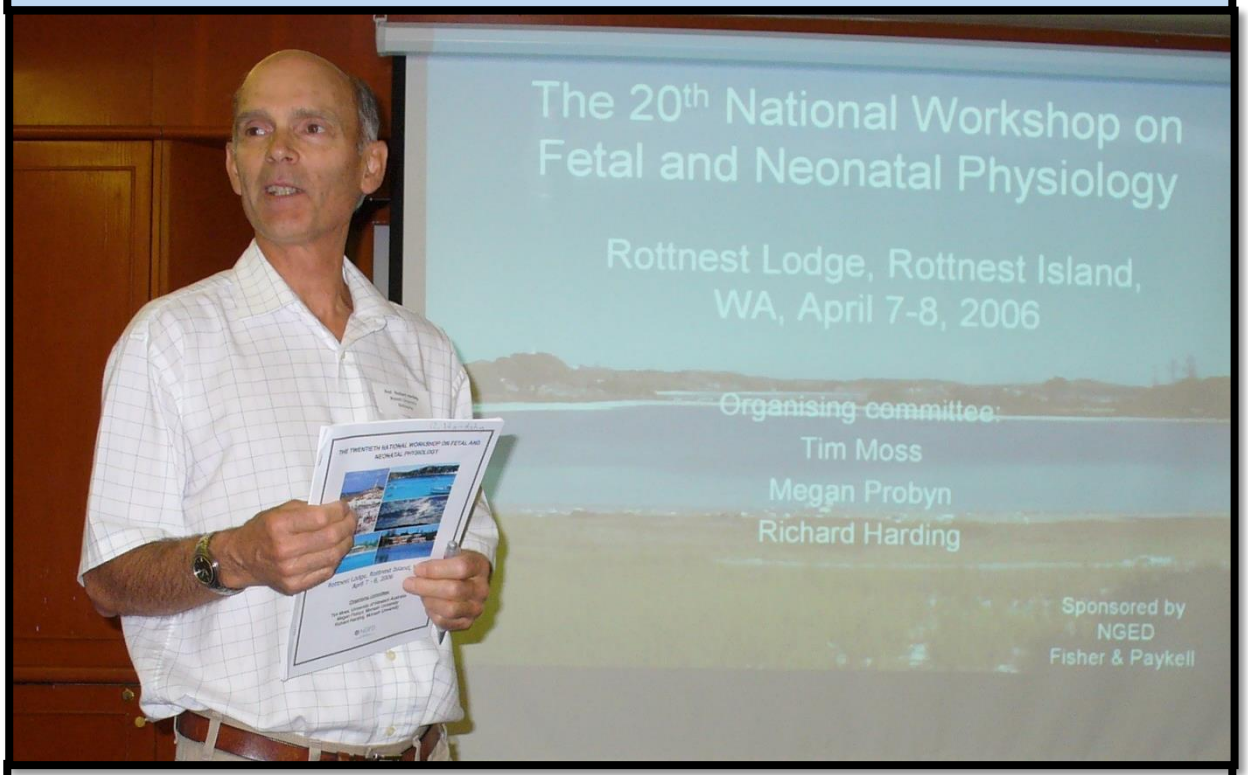
FETAL AND NEONATAL WORKSHOP
OF AUSTRALIA AND NEW ZEALAND
30th Annual Meeting

Festschrift in Honour of Professor Richard Harding



19 - 21 May, 2016
Peppers Blue on Blue Resort
Magnetic Island, QLD

2016 Organising Committee

Principal Organising Committee
Dr Robert De Matteo
Prof John Bertram

Local Organising Committee
Dr Yoga Kandasamy
Dr Barbara Lingwood

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Programme Outline - 2016

Peppers Blue on Blue Resort
Nelly Bay, Magnetic Island, QLD

WEDNESDAY 18TH MAY	
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THURSDAY 19TH MAY	
8.30am-9.30am	Registration
9.30am-11.03am	Session 1
11.03am-11.30am	Morning Tea
11.30am-1.27pm	Session 2
1.30pm-2.15pm	Lunch
2.15pm-3.33pm	Session 3
3.33pm-4.00pm	Afternoon Tea
Free time	

FRIDAY 20TH MAY	
8.45am-9.15am	Registration
9.15am-11.02am	Session 4
11.02am-11.30am	Morning Tea
11.30am-1.03pm	Session 5
1.03pm-2.00pm	Lunch
2.00pm-3.25pm	Session 6
3.25pm-3.55pm	Afternoon Tea
3.55pm-5.30pm	Session 7
6.30pm-11.30pm	Dinner Boardwalk Restaurant & Bar

SATURDAY 21ST MAY	
9.30am-10.00am	Registration
10.00am-11.45am	Session 8
11.45am-12.15pm	Morning Tea
12.15pm-1.38pm	Session 9
1.38pm-2.30pm	Lunch
2.30pm-3.38pm	Session 10
3.*38pm-4.00pm* *Presentation of student prizes, FNW 2017, Close of Workshop*	

FNWANZ Scientific Programme - 2016

DAY 1 - THURSDAY 19th MAY

Registration: 8.30am-9.30am

**=Short talks; U=Undergraduate, Hons=Honours, E=Early PhD, L=Late PhD, ECR=Early Career Researcher*

Session 1: Chairs – Roger Smith and Beth Allison			
9:30	**A1**	Alan Bocking	The microbiome and perinatal health: the next frontier?
10:00	**A2**	Roger Smith	Understanding uterine transformation in human labour
10:12	**A3**	Amanda Vrselja (E)	Effects of intrauterine inflammation on cardiac growth and maturation in preterm lambs
10:24	**A4**	Christine Astell (L)	Antenatal and postnatal influences on diaphragm function in preterm lambs
10:36	**A5**	Lauren Kerr (ECR)	Improving the aeration of non-uniformly aerated lungs
10:48	General discussion		

Morning tea: 11.03am-11.30am

Session 2: Chairs – Laura Bennet and Mary Tolcos			
11:30	**A6**	Dan Rurak	A postulated biological mechanism for the increased risk of stillbirth and other adverse birth outcomes with advancing gestational age
12:00	**A7**	Robert Galinsky	Connexin hemichannel blockade improves survival of striatal GABAergic neurons after fetal cerebral hypoxia ischaemia
12:12	**A8**	Alistair Gunn	Non-additive neuroprotection with delayed cerebral hypothermia and recombinant human erythropoietin infusion after prolonged cerebral ischemia in near-term fetal sheep
12:24	**A9**	Stephanie Miller (L)	Hypoxia-induced neonatal seizures are independently associated with reduced $GABA_A$ α3 subunit expression
12:36	**A10**	Kate Goasdoue (E)	A proposed study for investigation of the blood-brain barrier in neonatal seizures and hypoxic ischaemic encephalopathy
12:48	**A11**	Kirat Chand (ECR)	Expression of inflammatory mediators after hypoxic-ischemic brain injury in a neonatal pig model
1:00	**A12**	Riana Samuel (Hons)	Understanding the association between seizures and neuronal degeneration following birth asphyxia, and the neuroprotective potential of umbilical cord blood stem cells
1:12	General discussion		

Lunch: 1.30pm-2.15pm

Session 3: Chairs – Nicolette Hodyl and Leo Leader			
2:15	**A13**	Rosemary Horne	Sleeping like a baby – is this really a good thing?
2:35	**A14**	Dawn Elder	24 – hour oxygen saturation recordings at discharge in preterm infants – prevalence of intermittent hypoxia and comparison of different methods of editing data
2:47	**A15**	Helena Parkington	Potassium channel dysfunction in failed human labour
2:59	**A16**	Nadia Bellofiore (E)	Pre-menstrual Mouse? The common spiny mouse as a model for PMS
3:11	***A17**	Jane Pillow	Antenatal inflammation ablates early postnatal development of circadian rhythm in preterm fetal lambs
3:18	General discussion		
Afternoon tea: 3.33pm - End Day 1			

DAY 2 - FRIDAY 20th MAY

Registration: 8.45-9.15am

**=Short talks; U=Undergraduate, Hons=Honours, E=Early PhD, L=Late PhD, ECR= Early Career Researcher*

Session 4: Chairs – Michael Stark and Jon Hirst			
9:15	**A18**	Abby Fowden	Glucocorticoid programming of intrauterine development
9:45	**A19**	Jack Darby (E)	Preterm birth coupled with antenatal glucocorticoid treatment increases cardiac MR and 11β-HSD1 in adult life
9:57	**A20**	Ishmael Inocencio (E)	Nitric oxide as a potential therapeutic against cardiovascular dysfunction in fetal growth restricted fetuses
10:09	**A21**	Tim Cole	Glucocorticoids regulate cell type-specific pathways in the interstitial mesenchyme and epithelial compartments of the mammalian fetal lung
10:21	**A22**	Jia Yin Soo (L)	The effect of substrate supply on fetal hepatic gene expression of drug transporters and drug metabolising enzymes
10:33	***A23**	Tamás Zakár	Epigenetic regulation of CRH expression in human trophoblasts
10:40	***A24**	Emma Buckels (L)	Developing a method to visualise the endocrine pancreas using whole-slide imaging
10:47	General discussion		

Morning tea: 11.02am-11.30am

Session 5: Chairs – Jane Pillow and Alistair Gunn			
11:30	**A25**	Gert Maritz	Effect of maternal nicotine exposure on lung development: An overview
12:00	**A26**	Ali Hani (E)	Neonatal hyperoxia affects macrophage phenotype in mice: effectiveness of mesenchymal stem cell therapy
12:12	**A27**	Ruhan Kruger (U)	The effect of maternal whooping cough vaccination on fetal development and postnatal behaviour in spiny mice
12:24	**A28**	Stacey Ellery (ECR)	A pilot study of maternal creatine treatment in primate pregnancy- protecting the fetus from late gestation brain injury
12:36	**A29**	Yan Yee Chan (Hons)	Optimising the dose of erythropoietin required to prevent ventilation-induced brain injury
12:48	General discussion		

Lunch: 1.03pm-2.00pm

Session 6: Chairs – Graeme Polglase and Kathy Gatford			
2:00	**A30**	Tim Moss	Preterm lung maturation: a cautionary tale and a new direction
2:20	**A31**	Tanzila Mahzabin (L)	The influence of antenatal steroid on preterm sheep diaphragm
2:32	**A32**	Amy Wooldridge (L)	Effects of maternal asthma on the fetal immune system
2:44	***A33**	Paris Papagianis (E)	The effect of postnatal steroids on the lung structure of ventilated preterm lambs exposed to chorioamnionitis
2:51	**A34**	Julia Shaw (L)	Maternal administration of progesterone: effects on neurosteroidogenesis and neurodevelopment
3:03	***A35**	Madison Paton (E)	Brain inflammation in preterm fetal sheep to examine the benefits of umbilical cord blood and cord tissue stem cell therapies
3:10	General discussion		

Afternoon tea: 3.25pm-3.55pm

Session 7: Chairs – Barbara Lingwood and Karen Moritz			
3.55	**A36**	Mary Wlodek	Cardiorenal and metabolic risk for offspring born small: impact of lifestyle and its consequences on the next generation
4:05	**A37**	Jane Black	Consequences of preterm birth on the immature renal and cardiovascular systems
4:25	**A38**	Ian Wright	Hydrogen sulphide production capacity in the perinatal heart
4:37	**A39**	Sarah Walton (L)	Prenatal hypoxia combined with a high-salt diet increases risk of renal and cardiovascular impairments in adult mice
4:49	**A40**	Yvonne Eiby	Pilot trial of early blood transfusions for supporting cardiovascular function and cerebral oxygen delivery in preterm piglets
5:01	**A41**	John Bertram	Maternal low protein diet leads to low podocyte endowment
5:13	General discussion		
5:30	End Day 2		
6.30pm – Dinner – Board Walk Restaurant and Bar			

DAY 3 – SATURDAY 21st MAY

Registration: 9.30-10.00am

**= Short talks; U=Undergraduate, Hons=Honours, E=Early PhD, L=Late PhD, ECR= Early Career Researcher*

Session 8: Chairs – Julie Owens and Kelly Crossley			
10:00	**A42**	Peter Nathanielsz	The need for nonhuman primate studies to determine mechanisms of developmental programming
10:30	**A43**	Angela Cumberland (L)	The combination of IUGR and prenatal maternal stress changes developmental profiles in guinea pigs
10:42	**A44**	Kirsten McInerney (E)	Altered patterns of behaviour with increasing age in male offspring following perinatal stress
10:54	**A45**	Kathryn Gatford	The metabolic response to exercise is sex-dependent and altered in sheep offspring of placentally-restricted multi-fetal pregnancies
11:06	**A46**	Lara Bush (Hons)	The neuroprotective effects of umbilical cord blood stem cells in intrauterine growth restriction
11:18	**A47**	Mitchell Lock (E)	Expression of miR-133a and miR-15 family and their target genes in the fetus and 6 month old sheep heart in response to myocardial infarction
11:30	General discussion		

Morning tea: 11.45am-12.15pm

Session 9: Chairs – Frank Bloomfield and David Todd			
12:15	**A48**	Stuart Hooper	Non-invasive ventilation in the delivery room
12:35	**A49**	Marcus Davey	Extrauterine support for extreme prematurity
12:47	**A50**	Fiona Stenning (E)	Effects of oxytocin administration during physiological-based cord clamping on the cardiorespiratory transition at birth
12:59	**A51**	Joseph Smolich	Blunted sympathoadrenal activation with increased haemodynamic stability at birth in preterm lambs: immediate & delayed cord clamping
13:11	**A52**	Zeena Al-Obaidi (H)	Evaluating the relationship between diaphragm function and respiratory failure in preterm infants
13:23	General discussion		

Lunch: 1.38pm-2.30pm

Session 10: Chairs – Tamás Zakár and Ian Wright			
2:30	**A53**	Megan Wallace	Identifying the factors that regulate lung development
2:50	**A54**	Emily Cohen (L)	Growth restricted preterm neonates display compromised heart rate variability on the first postnatal day
3:02	***A55**	Keiji Suzuki	Ionized and total magnesium in the plasma in neonates - in comparison with calcium
3:09	***A56**	Donna Rudd	The effect of body weight on serum creatinine and cystatin C measurements in neonates
3.16	***A57**	Christiane Theda	GABA Receptor 1 gene methylation changes as an example of differential DNA methylation in blood of preterm versus term babies
3:23	General discussion		
3:45	**Presentation of Prizes**		
3:50	**Fetal and Neonatal Workshop 2017**		
4:00	**Close of Workshop**		

Fetal and Neonatal Workshop of Australia and New Zealand (FNWANZ)

Terms of reference

- The FNWANZ provides a forum for discussion of new ideas and presentation of experimental and clinical data in fetal and neonatal biology
- The FNWANZ aims to encourage discussion and establish collaborations between basic scientists and researchers from all disciplines of perinatal medicine
- The FNWANZ is an informal, multidisciplinary meeting with workshop-style presentations and discussion sessions from scientists and researchers from all disciplines of perinatal medicine
- The FNWANZ meetings consist of oral communications on completed studies, works in progress or planned studies

The Fetal and Neonatal Workshop gratefully acknowledges the financial support from;

MONASH University
Medicine, Nursing and Health Sciences
Department of Anatomy and Developmental Biology

PERINATAL SOCIETY of Australia & New Zealand
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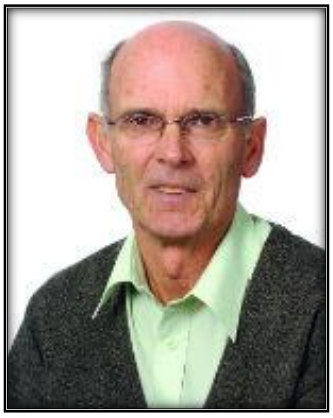
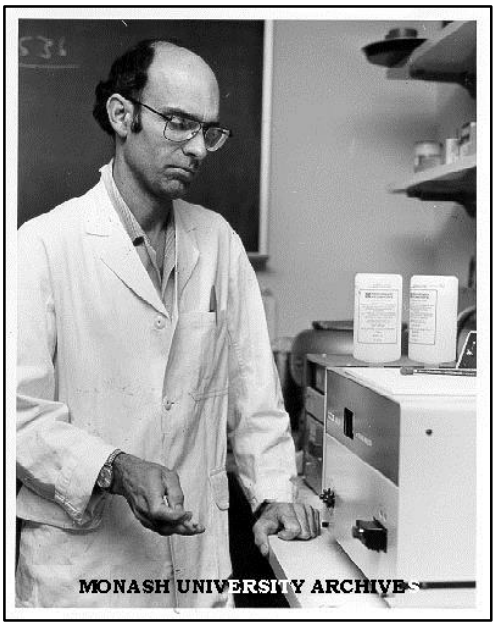


Emeritus Professor Richard Harding

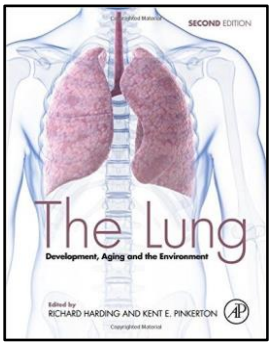
A short biography

I undertook my undergraduate studies (BSc, 1963-5) at the University of Melbourne, majoring in Physiology and Pharmacology. That led to me studying for my MSc (1966-7) at the Howard Florey Institute of Medical Research, investigating the effects of sodium concentration on smooth muscle contractility. In 1968 I moved to the University of Edinburgh to undertake a PhD on the central neural control of forestomach motility in sheep, graduating in 1971. After a short period of teaching in the Department of Physiology, University of Melbourne, I moved to a post-doctoral position at the Nuffield Institute for Medical Research, University of Oxford, in 1974 where I was first exposed to developmental physiology: my mentors at the Nuffield Institute were Paul Johnson and Geoffrey Dawes. After leaving Oxford in 1978 I moved to Brisbane where I was awarded a University of Queensland Research Fellowship to work in the Dept of Physiology and Pharmacology, as part of Geoff Thorburn's developmental physiology group.



In 1981 I moved with Geoff Thorburn's group to Monash University, Dept of Physiology, where I formed my own research group continuing my work on the prenatal regulation of lung development and fetal fluids, initially as an NHMRC Research Fellow and finally as a Senior Principal Research Fellow and Professorial Fellow (in 1995 and 1996 respectively). In 1992 I was awarded a DSc by Monash University for published research in developmental physiology. While at Monash I began numerous productive collaborations, such as those with Sandra Rees (investigating causes of fetal brain injury), Gert Maritz (lung development) and Jane Black (perinatal factors affecting cardiovascular development). In 2006, at the invitation of John Bertram, I moved to the Dept of Anatomy & Developmental Biology at Monash in order to enhance my group's research. Here I was recently appointed Emeritus Professor following formal retirement at the end of 2015.

Since 1980, my research group has been continuously supported by grants from the NHMRC, as well as a number of charitable organisations. During the course of my research career I have published 320 research papers including reviews and book chapters. I have also edited a number of books including “Textbook of Fetal Physiology” (with Geoff Thorburn), “Fetal Growth and Development” (with Alan Bocking), and most recently “The Lung: Development, Aging and the Environment” (with Kent Pinkerton). A second edition of the latter book has just been published.

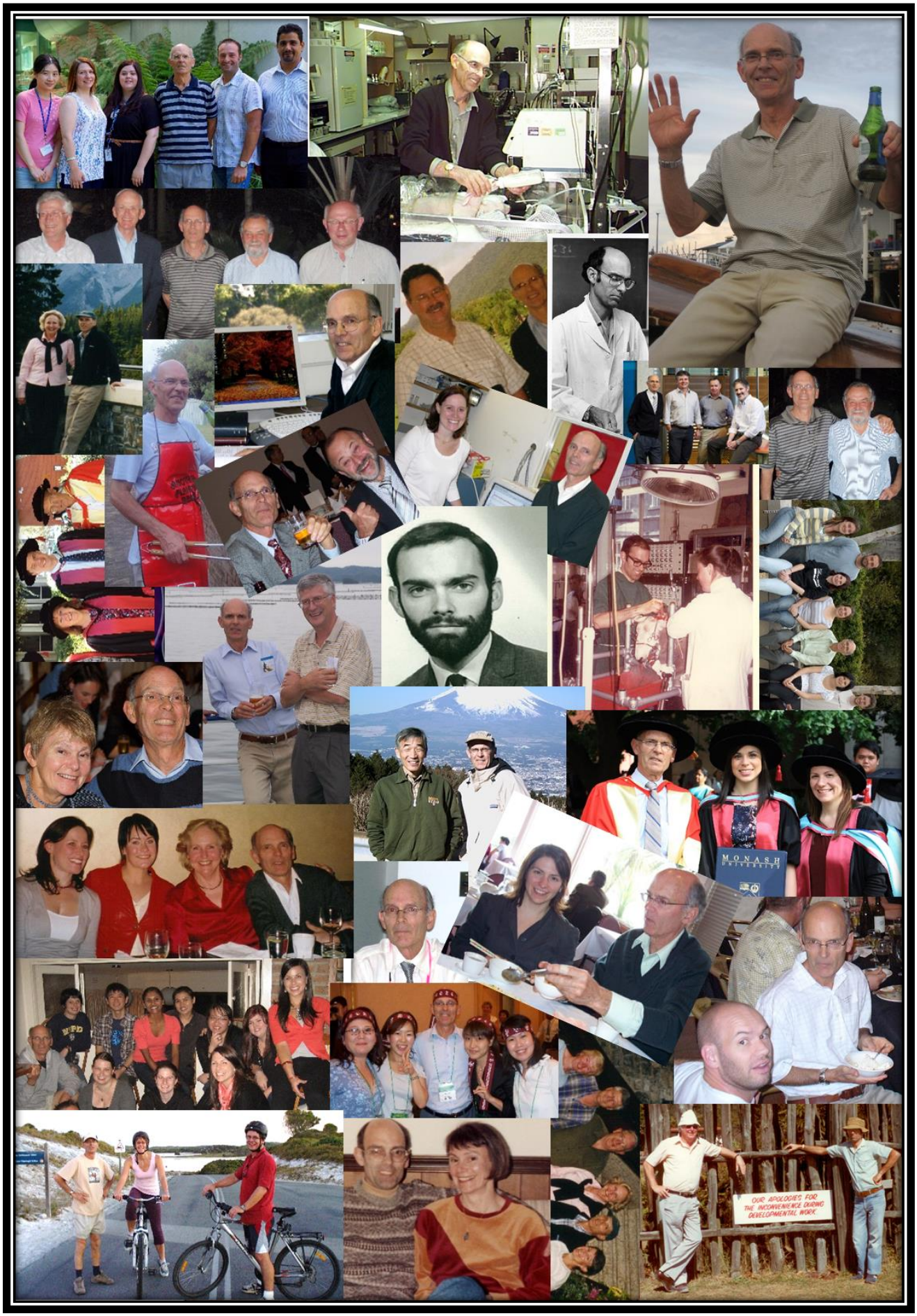


An enjoyable aspect of my career has been teaching and mentoring at all levels, from undergraduate to post-doctoral. One highlight of undergraduate teaching is the third-year course I initiated in Fetal and Neonatal Developmental Physiology which is still being run after 20+ years. In addition to supervising numerous honours and masters students, I have supervised 24 PhD students and have mentored several post-doctoral fellows. In recognition of my supervisory duties I was awarded the Vice Chancellor’s Award for Postgraduate Supervision in 2004.

In recent years our work has focussed on understanding the impact of fetal exposure to environmental factors such as hypoxia, IUGR, intrauterine inflammation and alcohol on organ development and later function. We have studied the effects of these fetal exposures on the fetus, newborn and adult, focussing on the lungs, brain, kidneys, heart and liver. More recently, we have focussed on the effects of neonatal hyperoxia and preterm birth on development. For the last 10 years my research group has been supported by two successive NHMRC Program Grants for studies aimed at improving outcomes for preterm babies. The first program grant was selected as being one of the 10 best NHMRC-funded research projects in 2012, in terms of its outcomes and significance in relation to improving health outcomes for Australians.

Prof Richard Harding, Prof Stuart Hooper, A/Prof Tim Cole, Prof Peter Davis

One of my most rewarding achievements was helping to found the Fetal and Neonatal Workshop in 1986, and to oversee its continuation as an annual event since then. Not only has the Workshop provided an important venue for the critical presentation of research findings in perinatal biology, it has led to many scientifically rewarding collaborations and friendships; and it has enabled participants to visit many interesting places in Australia and New Zealand.


MONASH
OUR APOLOGIES FOR THE INCONVENIENCE DURING DEVELOPMENTAL WORK

Biosketch – Keynote Guests

Professor Jane Black

Prof Jane Black is the Deputy Head of Department (Teaching and Research Training) and Head of the Cardiovascular and Renal Developmental Programming Laboratory in the Department of Anatomy & Developmental Biology at Monash University.

Prof Black has a strong background in cardiovascular cell biology. Her current research focuses on the effects of perturbations in early life on the development of the heart and kidney and the long-term consequences. Her research group has made major contributions to understanding the mechanisms of the developmental programming of cardiovascular and renal disease. Her research group is leading research worldwide into the effects of preterm birth on the heart and kidney. In particular, her studies have provided seminal insight into the maladaptive remodelling of the heart and great vessels in response to preterm birth. Her research group have also shown that preterm birth is often associated with glomerular abnormalities in the kidneys of preterm infants which have the potential to impact on short-term and long-term renal function.

Prof Black is internationally recognised for her morphological and stereological analyses of the developing kidneys, heart and vasculature.

Professor Alan Bocking

Dr. Alan Bocking is a Professor in the Departments of Obstetrics and Gynaecology and Physiology at the University of Toronto and Senior Clinician Scientist at the Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, Canada. He is the former Gordon C. Leitch Chair of the University of Toronto, Department of Obstetrics and Gynaecology and Chief of the Department of Obstetrics and Gynaecology at Mount Sinai Hospital, the University Health Network and Women's College Hospital (2003 – 2013). Dr. Bocking's main areas of study are the mechanisms underlying infection–mediated preterm labour including the role of the vaginal microbiome. His other research interests include understanding the mechanisms underlying Fetal Alcohol Spectrum Disorder as well as creating research Infrastructures to study the Developmental Origins of Health and Disease (DOHaD). He currently chairs the Steering Committee for the Ontario Birth Study which is an open longitudinal pregnancy cohort based at Mount Sinai Hospital, Toronto and has published over 100 peer-reviewed articles on his research findings. He was the Founding Director of the Academic Model for Provision of Access to Health Care – Reproductive Health Program, (AMPATH-RH) from 2008 – 2014 which partners with Moi University School of Medicine in Eldoret, Kenya. Dr. Bocking is currently the Vice-Chair of the Board of the Maternal Infant, Child and Youth Research Network (MICYRN), and Vice-President of the Board of the Molly Towell Perinatal Research Foundation. He is a member of the Advisory Board of the CIHR Institute of Human Development, Child and Youth Health and currently serves as Scientific Advisor for the Canadian Fetal Alcohol Spectrum Disorder (Can FASD) Research Network.

Professor Abby Fowden

Abigail Fowden is Professor of Perinatal Physiology in the Department of Physiology, Development and Neuroscience and Head of the School of the Biological Sciences at the University of Cambridge. She was an undergraduate at Girton College and graduated with a first class degree in Physiology in 1975. She obtained her PhD from the University of Cambridge in 1979 and immediately joined the staff of the Department of Physiology as a demonstrator. Since then, she has held positions as a University Lecturer and Reader before being promoted to a personal chair in 2002. She obtained the ScD degree in 2001 and was awarded the Joan Mott Prize of the Physiological Society for her research in 2008. Her research interests are in the factors controlling feto-placental growth and development during late pregnancy. The aims of her research are two-fold: first, to determine how hormones and other environmental cues regulate feto-placental development, and secondly, to establish how our experiences during early life alter the risk of degenerative diseases in adulthood. She is also a Professorial Fellow at Girton College, Cambridge.

Professor Stuart Hooper

Professor Stuart Hooper is a NHMRC Principal Research Fellow and Director of the Ritchie Centre, Hudson Institute of Medical Research and Research Director for Department of Obstetrics and Gynecology, Monash University. He is a fetal and neonatal physiologist whose research focuses on fetal and neonatal lung development and its transformation into a functional gas-exchange organ at birth. His research focuses on; (1) factors regulating normal and abnormal growth of the lung, (2) the cardiovascular and respiratory transition at birth and (3) how respiratory support of very preterm infants can be improved to facilitate their transition and avoid injury to the lungs and brain. Prof Hooper leads a multi-disciplinary research team that has pioneered the use of phase-contrast X-ray imaging to image the entry of air into the lungs at birth. Using this technology they have made significant discoveries, including (i) the processes driving lung aeration at birth, (ii) the relationship between lung aeration and the increase in pulmonary blood flow and (iii) describing lung motion throughout a breath. As such, they expect to make significant advances in understanding the physiology underpinning the transition to newborn life at birth in both term and preterm infants.

Professor Rosemary Horne

Professor Rosemary Horne is a Senior Principal Research Fellow and heads the Infant and Child Health research theme within the Ritchie Centre, Hudson Institute of Medical Research and Department of Paediatrics, Monash University. Her research interests focus on numerous aspects of sleep in infants and children. Rosemary has published more than 150 scientific research and review articles. She is Chair of the Physiology working group of the International Society for the Study and Prevention of Infant Deaths and the SIDS and Kids Australia National Scientific Advisory Group, a Director of the International Paediatric Sleep Association, and is on the editorial boards of the Journal of Sleep Research, Sleep and Sleep Medicine.

Professor Gert Maritz

Prof Maritz commenced work at the University of Western Cape, South Africa in 1975, as lecturer in the Department of Physiology and has received ad hominem promotions to Senior Professor, in the Department of Medical Biosciences. Prof Maritz was awarded an Emeritus Professor position in 2013. Prof Maritz has served on numerous departmental, faculty, senate and council committees at UWC, and also held the position of departmental chair. While working at UWC he completed his MBA degree.

Prof Maritz has served as an external examiner of physiology at Medical Schools of University of Stellenbosch, Witwatersrand, Pretoria, Bloemfontein and the courses at the Universities of Stellenbosch and Witwatersrand. Prof Maritz has also served as an external examiner for Masters and Doctoral degrees, both nationally and internationally.

Prof Maritz's research has focused on the effect of in utero compromises on lung development in offspring through to adulthood. A major interest has been the effect of nicotine exposure, during gestation and lactation, on lung development in the offspring. He has collaborated with researchers mostly at Monash University, Australia, especially with Prof Richard Harding, during which time they studied the effect of intrauterine growth retardation, placental insufficiency and inadequate nutrient supply on lung development.

Prof Maritz has authored 77 peer reviewed articles, 7 book chapters and written several reviews. He has written articles for South African newspapers to inform the public of the dangers of smoking and nicotine, especially on the permanent adverse impact on the respiratory health of exposed offspring. He has been interviewed on this topic by several South African radio stations and wrote an article for distribution to schools to inform children and teachers about the harmful effects of smoking and nicotine on health in the long term. He has recently completed a book on the effect of grand-parental lifestyle on the health of their progeny.

A/Professor Tim Moss

A/Prof Tim Moss was supervised by Richard Harding for his Honours year in 1993, and during his PhD from 1994-1998. Richard helped Tim obtain a postdoctoral position at The University of Western Australia, and with the establishment of his independent research career. They received NHMRC project grants in 2000 and 2001, which provided the springboard for Tim's successful application for an NHMRC RD Wright Biomedical Career Development Fellowship in 2014 (Tim was the highest ranked applicant). When Tim had enough of Perth, Richard was the first to call, and invited him to return to Monash. Since then Tim and Richard have been coinvestigators on an NHMRC program grant. They continue to work together.

Tim has published 134 research papers, reviews and book chapters (20 with Richard). His work has received over 3700 citations (455 for publications with Richard). He has received over $21 million in research grant funding as CI ($9.4 million with Richard).

Tim's research is focused on understanding how exposure to infection or inflammation in utero alters development of the fetus to affect health after birth. His

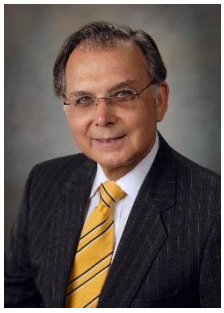
group is also investigating ways to treat or prevent inflammation and its effects in newborns.

Tim is a Board Member of the international Fetal and Neonatal Physiological Society and occupies senior positions in the Perinatal Society of Australia and New Zealand.

Tim learned to write from Richard, and he has gone on to become an accomplished scientific communicator. Tim has written for crikey.com, been interviewed on Melbourne's 3RRR FM and provided expert opinion for New Scientist. He trained in science communication at The Alan Alda Centre for Communicating Science at Stony Brooke University (USA).

Tim co-convenes BME3082 'Fetal and Neonatal Development', which is consistently ranked by students in the top 7% of all units taught at Monash University. He still remembers Richard's lectures in its precursor, PHY3082, which was convened by Richard when Tim was a 3rd-year undergraduate student.

Professor Peter Nathanielsz



Peter Nathanielsz obtained a Bachelor's, Medical degree, PhD and a Sc.D. from Cambridge University where he developed a lasting interest in understanding fetal physiology. He was a Fellow of St. Catharine's College for seven years and a lecturer in the Physiological Laboratory. In 1976 he set up Laboratory of Fetal Physiology at the University of California, Los Angeles. In 1982, he moved to College of Veterinary Medicine, Cornell University, Ithaca New York as the Director of the Laboratory for Pregnancy and Newborn Research. During the 1980s he undertook two exciting Antipodean Sabbaticals, one in Brisbane where he had the pleasure of working with Richard Harding and the other in Auckland with Mont Liggins. From 2002 – 2004 he was Director of the Center for Women's Health Research at New York University. In 2004 the Center relocated to the University of Texas Health Science Center at San Antonio and formed the Center for Pregnancy and Newborn Research. In 2004 he was appointed Distinguished Professor of Life Course Health at the University of Wyoming. He has retained his program at San Antonio.

Using fetal sheep stereotaxic lesions Peter Nathanielsz and Thomas McDonald were the first to show that the signals to start labor and delivery require viable fetal paraventricular nuclei.

His current interests are in comparative approaches to the study of life course health outcomes of developmental programming in rodents, sheep and nonhuman primates.

The NIH has continuously funded his research group since 1976. Funding has also been obtained from the March of Dimes, National Science Foundation, the European Union, the MRC and several independent foundations and organizations.

Professor Dan Rurak

Dan Rurak, MSc. D.Phil., University of British Columbia, Vancouver, B.C., Canada

Dr. Rurak is an Emeritus Professor of Obstetrics & Gynecology in the Faculty of Medicine, University of BC and a Senior Scientist in the Child & Family Research Institute in Vancouver, Canada. He received B.Sc. and M.Sc. degrees in Zoology from the University of British Columbia in 1968 and 1971, respectively. From 1972 to 1976, he completed doctoral training in perinatal physiology at the Nuffield Institute for Medical Research in Oxford, U.K. He followed this with postdoctoral studies in Oxford and taking up a position as Assistant Professor at UBC in 1978. His research interests include fetal cardiorespiratory physiology and CNS functions, fetal oxygenation, and maternal-fetal and neonatal drug disposition and effects. He has conducted both animal and human studies of drugs, including metoclopramide, diphenhydramine, labetalol, ritodrine, indomethacin, valproic acid, fluoxetine, paroxetine and sertraline. His current research interests include the fetal and postnatal effects of antenatal SSRI antidepressant exposure in human subjects, NIRS measurement of fetal oxygenation and changes in fetal oxygenation with advancing gestation. His research has resulted in a total of over 290 publications (papers and abstracts).

Dr Megan Wallace

Megan heads the Lung Development Research Group in the Ritchie Centre, Hudson Institute of Medical Research and is the Director of Medical Student Research for the Faculty of Medicine, Monash University.

Megan performed her Honours and PhD studies in the Department of Physiology, Monash University under the supervision of Prof. Richard Harding and Dr. Stuart Hooper, investigating the regulation of fetal lung liquid secretion and reabsorption. Her postdoctoral fellowship was at the Hospital for Sick Children in Toronto, Canada where she honed her skills in molecular biology and developed a knockout mouse for protein tyrosine phosphatase alpha that was published in Nature Genetics. Since she returned to Australia, her research has focussed on identifying the mechanisms regulating normal and abnormal lung development, and understanding the transition to air-breathing at birth. She has developed sophisticated molecular techniques, live-cell and X-ray imaging approaches, and intricate surgical procedures to manipulate, image and analyse lung development in sheep, rabbits, rats and mice.

Professor Mary Wlodek

Mary Wlodek is a Professor in the Department of Physiology, School of Biomedical Sciences, Faculty of Medicine, Dentistry and Health Sciences, The University of Melbourne. Professor Wlodek is also Associate Dean (Research) in the Faculty of Medicine, Dentistry and Health Sciences, The University of Melbourne. Professor Wlodek graduated with a BSc (Hons) and MSc from the University of Western Ontario, London, Canada. She was awarded her PhD in Physiology from Monash University, Australia and was supervised by Profs Richard Harding and Geoffrey Thorburn. She is a global leader in developmental origins physiological research and Head of the Fetal, Postnatal & Adult Physiology & Disease Laboratory. She is renowned for her successful experimental model that mimics human growth profiles, organ deficits and phenotypes observed in babies born small who are susceptible to adult diseases. Her laboratory is recognised as performing complex whole animal physiological studies exploring the adult, pregnancy and transgenerational consequences of being born small. Critical to translational outcomes is the incorporation of various innovative treatments and interventions including nutritional (cross-fostering, diet), exercise, pregnancy, transgenerational and impact of stress and alcohol during pregnancy.

Professor Wlodek's strong commitment to mentoring has also been recognised through prestigious awards, including the Faculty of Medicine, Dentistry and Health Sciences Excellence Award for Equity and Staff Development (2012), the University's James Angus Award for Outstanding Research Higher Degree Supervision and subsequently the Australian Government, Office for Learning & Teaching, Citation for Outstanding Contributions to Student Learning (2013).

Session 1

Chairs – Roger Smith and Beth Allison			
9:30	**A1**	Alan Bocking	The microbiome and perinatal health: the next frontier?
10:00	**A2**	Roger Smith	Understanding uterine transformation in human labour
10:12	**A3**	Amanda Vrselja (E)	Effects of intrauterine inflammation on cardiac growth and maturation in preterm lambs
10:24	**A4**	Christine Astell (L)	Antenatal and postnatal influences on diaphragm function in preterm lambs
10:36	**A5**	Lauren Kerr (ECR)	Improving the aeration of non-uniformly aerated lungs
10:48	General discussion		

The microbiome and perinatal health: the next frontier?

Alan D. Bocking

Departments of Obstetrics and Gynecology and Physiology, University of Toronto, Toronto, Canada.
abocking@mtsinai.on.ca

Background: The human microbiome has been the subject of much interest recently as it relates to health and disease. In this presentation, I will review the evidence in humans and animals that the microbiome in multiple sites including the oral cavity, GI tract and vagina plays an important role in ensuring a healthy pregnancy outcome for both the mother and fetus/neonate. Preterm birth (PTB), in particular, occurs in 10% of all pregnancies globally. Premature babies have a mortality rate 40 times higher than term infants. Approximately 25-30% of PTB can be attributed to intrauterine infection/inflammation and a disturbance of the vaginal microbiota as observed in bacterial vaginosis (BV) is associated with an increased risk of PTB. Treatment of preterm labor with antibiotics is largely ineffective, and probiotic lactobacilli have been proposed as a potential preventive therapy for BV and PTB. Specific experiments in pregnant mice and in humans to address this issue will be discussed.

Aims: 1) To assess the effect of *Lactobacillus rhamnosus* GR-1 (GR-1) and its supernatant (GR-1 SN) on the prevention of lipopolysaccharide (LPS)-induced PTB as well as systemic and intra-uterine cytokine and chemokine profiles in pregnant CD-1 mice.

2) To determine the effect of GR-1 and *L. reuteri* RC-14 on the cervico-vaginal cytokine profile and vaginal microbiota in pregnant women with an abnormal Nugent score.

Methods: 1) Pregnant mice were pre-treated with intra-peritoneal injections of GR-1 SN prior to intrauterine injection of LPS. The expression of cytokines and chemokines in the maternal plasma, amniotic fluid and intrauterine tissues were then measured.

2) A randomized, double blind placebo-controlled trial was conducted in which pregnant women with an abnormal Nugent score in their first trimester of pregnancy received orally either placebo or GR-1 and RC-14 for 12 weeks. Their cervico-vaginal cytokine profile and vaginal microbiota were then determined.

Results: 1) Pre-treatment with GR-1 SN reduced LPS-induced PTB by 40% and was associated with a decrease in pro-inflammatory cytokines and chemokines with no change in anti-inflammatory cytokines when compared to LPS-treated control animals. Parallel changes in pro- and anti-inflammatory cytokines were seen in intrauterine tissues and amniotic fluid.

2) Oral GR-1 and RC-14, for 12 weeks, did not change the vaginal cytokine profile or microbiota of pregnant women with an abnormal Nugent score. In keeping with other studies, the vaginal microbiota was remarkably stable across gestation in both the placebo and probiotic treatment groups.

Conclusions: We conclude that *L. rhamnosus* GR-1 supernatant acting through an alteration in systemic immune responses is able to reduce the risk of PTB in animals and is worthy of further investigation. In addition, although probiotic lactobacilli do not alter the vaginal microbiome in healthy low-risk women, studies are needed in women at risk based on clinical criteria to further evaluate its potential role in preventing inflammation-associated conditions during pregnancy, including PTB.

Understanding uterine transformation in human labour

Roger Smith, Cheng Chan, Tamas Zakar

Mothers and Babies Research Centre, Hunter Medical Research Institute, University of Newcastle, Newcastle, NSW, Australia.
Roger.Smith@newcastle.edu.au

Background: It has been difficult to develop a model of how the human uterus transforms into a contractile organ at term when only a single sample of myometrial tissue can be obtained at caesarean section.

Aims/Hypothesis: To use mathematical modelling to establish a biochemical pathway to uterine transformation to a contractile phenotype in the human uterus at term.

Methods: Myometrial samples were collected at term caesarean section from 30 women prior to the onset of labour and 30 women in active labour. Samples were subjected to quantitative PCR for 30 genes previously identified by subtractive hybridisation as being increased with labour. RNAseq was performed on 6 labouring and 6 non-labouring myometrial samples.

Results: Quantitative PCR data on the 60 samples was ranked for each of the 30 genes and a mean rank was generated for each sample on the pathway to myometrial transformation into a contractile phenotype. Increases in these genes were confirmed with RNAseq. The results indicated a clear hierarchy compatible with an epigenetically mediated transformation event following RelA interactions with steroid receptors and modulators of transcription and translation efficiency such as ELAV1/HuR and CRSP6. The changes in the myometrium appear secondary to endocrine events in the placenta including increased synthesis of corticotrophin releasing hormone.

Conclusions: Understanding the pathway to uterine transformation into a contractile phenotype allows the identification of many new targets for therapeutics to arrest or initiate uterine contractile behaviour.

Effects of intrauterine inflammation on cardiac growth and maturation in preterm lambs

Amanda Vrselja[1], J Jane Pillow[2, 3], P B Noble[2], S Ahmadi-Noorbakhsh[2] & M Jane Black[1]

[1]Department of Anatomy and Developmental Biology, Monash University, Clayton, Australia; [2]School of Anatomy, Physiology & Human Biology; and [3]Centre for Neonatal Research and Education, School of Paediatrics and Child Health, University of Western Australia, Perth, Australia.
amanda.vrselja@monash.edu

Background: Intrauterine inflammation, a common antecedent of preterm birth, may impact on the development and maturation of the heart, particularly cardiomyocyte development. Cardiomyocytes normally mature and terminally differentiate during late gestation, reducing their proliferative capacity. A high proportion of cardiomyocytes are immature at birth in the preterm heart. Consequently, cardiomyocyte number and differentiation are likely influenced by exposure to intrauterine inflammation in the preterm subject.

Aims/Hypothesis: To investigate the effect of intrauterine lipopolysaccharide (LPS) exposure, a model of intrauterine inflammation, on postnatal cardiac growth and maturation in preterm lambs.

Methods: Date-mated merino ewes were randomised to receive either an intra-amniotic injection of LPS (4mg, E coli 055:B5, Sigma Aldrich) to induce an intrauterine inflammatory response, or saline as an experimental control, 48 h prior to preterm delivery. Lambs were delivered preterm at 128 d gestation and managed postnatally in the Preclinical Intensive Care Research Unit (PICRU). At 7 d postnatal age lambs were euthanised and the hearts excised and perfusion fixed. The perfusion fixed hearts were sampled randomly and embedded in either glycolmethacrylate or paraffin for analysis of cardiac morphology, including cardiomyocyte number, nuclearity and proliferation, and cardiac extracellular matrix deposition.

Results: Analysis of cardiac tissue is currently in progress.

Conclusions: It is expected LPS-exposed preterm lambs will exhibit accelerated cardiomyocyte maturation and this will, therefore, negatively impact cardiomyocyte endowment in the heart. The findings from this study will be presented at the Workshop.

Antenatal and postnatal influences on diaphragm function in preterm lambs

Astell C J[1], Bakker A T[1], Ahmadi-Noorbakhsh S[1], Noble P B[1], Pillow J J[1,2], Pinniger G J[1]

[1]School of Anatomy, Physiology and Human Biology, University of Western Australia, Perth, Australia; [2] Centre for Neonatal Research and Education, School of Paediatrics and Child Health, University of Western Australia, Perth, Australia.
Christine.Astell@research.uwa.edu.au

Background: Antenatal exposure of the fetus to intra-amniotic (IA) lipopolysaccharide (LPS) impairs contractile function and induces atrophy of the preterm lamb diaphragm at birth for up to 21 d after exposure[1]. Whether diaphragm dysfunction following exposure to *in utero* inflammation persists into postnatal life, after the onset of spontaneous ventilation, is unknown. Diaphragm dysfunction resulting from inflammation and immaturity may contribute to respiratory insufficiency and the need for ventilator support in the preterm infant.

Aim: To determine the effects of antenatal inflammation induced by lipopolysaccharide on postnatal diaphragm function in preterm lambs.

Methods: Lambs were exposed to intra-amniotic (IA) LPS (4 mg, E coli 055:B5, Sigma Aldrich) or saline as control 48 h prior to premature delivery at 128 d gestational age (GA, term = 150 d). Lambs were managed in an intensive care environment then euthanized at 7 d postnatal age (135 d postconceptional age). End-point, fetal control lambs were delivered at 135 d GA and euthanized immediately prior to initiation of spontaneous ventilation. Following euthanasia, longitudinal strips from the right hemi-diaphragm were dissected for *in vitro* assessment of contractile function.

Results: IA LPS exposure did not affect contractile function of the preterm diaphragm at 7 d postnatal age ($P > 0.05$). Interestingly, diaphragm function changed significantly with 7 d of postnatal development. Diaphragm from LPS naïve and LPS treated lambs had a greater maximum intrinsic force capacity ($P < 0.001$), contracted faster ($P < 0.001$), relaxed faster ($P = 0.007$), produced lower twitch force ($P < 0.001$) and less relative force at low stimulation frequencies twitch – 40 Hz ($P < 0.05$) compared to the 135 d non-breathing fetal controls.

Conclusions: LPS-induced diaphragm weakness at birth after a 2 d LPS exposure does not persist after one week of postnatal life in the preterm lamb. Our results indicate that postnatal events rather than antenatal events are the primary determinants of diaphragm function at one week postnatal age. Differences in the contractile properties of LPS naïve and LPS treated diaphragm compared to fetal control diaphragm are consistent with a shift in fibre type proportions from neonatal to adult phenotype and acceleration in diaphragm development with one week of postnatal life. We hypothesise that acceleration in diaphragm development results from the onset of spontaneous breathing.

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1 - Karisnan K, Bakker A J, Song Y, Noble P B, Pillow J J, Pinniger G J. Gestational age at initial exposure to in utero inflammation influences the extent of diaphragm dysfunction in preterm lambs. Respirology. 2015; 20(8):1255-62.

Improving the aeration of non-uniformly ventilated lungs

Kerr LT[1], Kitchen MJ[2], Lee KL[2], Wallace MJ[1], Lista G[3], Cavigioli F[3], Crawshaw JR[1] and Hooper SB[1]

[1]Hudson Institute of Medical Research; [2]Department of Physics and Astronomy, Monash University, Melbourne, Australia; [3]Department of Paediatrics, V. Buzzi Hospital, Milan, Italy.
Lauren.Kerr@Hudson.org.au

Background: Infants born preterm have difficulty transitioning to newborn life due to lung immaturity and medical intervention may be necessary. Intervention can involve intubation with an endotracheal tube and mechanical (conventional) ventilation; however, incorrect endotracheal tube placement can result in non-uniform lung aeration. Two strategies known to improve lung aeration are: administration of surfactant; and ventilation with a sustained inflation (prolonged initial inflation; SI).

Aim: To determine the optimal strategy to ventilate non-uniformly aerated lungs of a newborn rabbit.

Methods: We utilised phase contrast X-ray imaging to visualise lung aeration of newborn preterm rabbits. The kittens initially had one lung ventilated, then both lungs. We examined different strategies (conventional ventilation (CV), SI and surfactant administration) and measured functional residual capacity (FRC) and peak inflation pressure (PIP) as indicators of lung aeration.

Results: CV did not significantly improve the FRC or PIP of the non-aerated or aerated lung. An SI did significantly improve the FRC and PIP of the unaerated lung (p=0.025, p=0.022, respectively), however there was no benefit in these parameters to the aerated lung. Surfactant significantly improved the FRC and PIP in both the unaerated lung (p=0.002 for each) and aerated lung (p=0.002, p=0.048, respectively).

Conclusions: Conventional ventilation was ineffective at improving the aeration of non-uniformly aerated lungs. A sustained inflation improved the aeration of a non-aerated lung. However, surfactant was required to uniformly aerate both the aerated and non-aerated lung.

Session 2

Chairs – Laura Bennet and Mary Tolcos			
11:30	**A6**	Dan Rurak	A postulated biological mechanism for the increased risk of stillbirth and other adverse birth outcomes with advancing gestational age
12:00	**A7**	Robert Galinsky	Connexin hemichannel blockade improves survival of striatal GABAergic neurons after fetal cerebral hypoxia ischaemia
12:12	**A8**	Alistair Gunn	Non-additive neuroprotection with delayed cerebral hypothermia and recombinant human erythropoietin infusion after prolonged cerebral ischemia in near-term fetal sheep
12:24	**A9**	Stephanie Miller (L)	Hypoxia-induced neonatal seizures are independently associated with reduced $GABA_A$ $\alpha 3$ subunit expression
12:36	**A10**	Kate Goasdoue (E)	A proposed study for investigation of the blood-brain barrier in neonatal seizures and hypoxic ischaemic encephalopathy
12:48	**A11**	Kirat Chand (ECR)	Expression of inflammatory mediators after hypoxic-ischemic brain injury in a neonatal pig model
1:00	**A12**	Riana Samuel (Hons)	Understanding the association between seizures and neuronal degeneration following birth asphyxia, and the neuroprotective potential of umbilical cord blood stem cells
1:12	General discussion		

A postulated biological mechanism for the increased risk of stillbirth and other adverse birth outcomes with advancing gestational age

Dan Rurak, K.S. Joseph

Child & Family Research Institute, University of British Columbia, Vancouver, Canada.
drurak@cw.bc.ca

Background: Population studies in humans using the fetus at risk approach have demonstrated an increased risk of stillbirth and IUGR with advancing GA, with the risks occurring earlier and being greater in twin compared to singleton pregnancies. This suggests that the constraints on fetal growth and survival increase with advancing GA.

Hypothesis: The limiting factor is oxygen, because it is continuously needed, there are no alternatives, there are limited O_2 stores in the fetus and M to F O_2 transfer depends primarily on maternal and fetal placental blood flows.

Methods: Literature search for relevant data in support of the hypothesis.

Results: Although absolute uterine blood flow increases with advancing GA, when normalized to fetal weight, it falls progressively in human, sheep and several other species. The same occurs with umbilical blood flow (Qum) in human and sheep, and in the latter species this decrease continues when GA is prolonged by fetal hypophysectomy or adrenalectomy. These decreases in flow result in a decrease in fetal vascular Po_2, which has been demonstrated in human and sheep, associated with an increase in Pco_2 and decrease in pH. The decrease in Qum and fetal Po_2 decreases fetal O_2 delivery, which occurs in human and sheep. Moreover, in sheep there is a decrease in weight-normalized O_2 consumption between 75 and 135 d. The decrease in O_2 demands may be at least in part the result of 2 processes: a decrease in fetal motility (fetal breathing and body movements) and decrease in growth rate with advancing GA, both of which occur in humans and sheep. Moreover in individual fetal lambs, the GA at which body movements began to decrease (mean = 91.9 d) is linearly related to the GA at which fetal growth rate declines (mean = 113.1 d), suggesting that these 2 processes may be causally related. The failure of placental blood flows to keep pace with fetal growth may simply be due to an inability of the maternal and fetal cardiovascular systems to supply the placenta above a certain limit. Mechanistically, it may involve the GA-related changes in the pro- and anti-angiogenic factors involved in placentation, which in pregnant women are released into the maternal circulation and can be measured. In normal human pregnancy, the ratio of placental growth factor (PlGF)/ soluble Fms-like tyrosine kinase receptor (sFlt-1)+soluble endoglin concentrations, which is a measure of angiogenic activity, increases until ~27 wks GA and then declines progressively until term. *In vitro* studies of cultured trophoblasts have indicated the hypoxia decreases PlGF and increases sFlt-1 release, suggesting that the progressive fall in fetal oxygenation with advancing GA may be involved in the change in pro- and anti-angiogenic factors. The decrease in fetal motility with advancing GA may be due to the increase in fetal plasma PGE_2 concentration, which begins at ~120 days and is due to the prepartum rise in fetal cortisol. In addition, the fetal plasma levels of neurosteroids, which suppress fetal arousal, increase progressively with advancing GA. The prepartum cortisol rise also inhibits fetal growth. This cortisol rise is in part due to increased adrenal sensitivity to ACTH. It has been suggested that this maturation is in part due to the transient episodes of fetal hypoxemia that result from antepartum uterine contractions and episodic fetal activity. It could also involve the GA-related progressive fall in fetal vascular Po_2. Overall these results suggest a coordinated set of mechanisms that underlie the fetal responses to the progressive decrease in fetal O_2 delivery with advancing GA, with the progressive hypoxemia and transient hypoxemic episodes being central elements.

Connexin hemichannel blockade improves survival of striatal GABAergic neurons after fetal cerebral hypoxia ischaemia

Robert Galinsky[1,2], Joanne O Davidson[1], Colin R Green[3], Laura Bennet[1], Alistair J Gunn[1]

[1]The Department of Physiology, The University of Auckland, New Zealand; [2]The Ritchie Centre, Hudson Institute of Medical Research, Victoria, Australia; [3]The Department of Ophthalmology, The University of Auckland, New Zealand.
r.galinsky@auckland.ac.nz

Background: Basal ganglia damage after asphyxia at term remains common and is closely associated with later cerebral palsy. After asphyxia, connexin hemichannels can open, leading to calcium influx and release of ATP and glutamate, resulting in neuronal death.

Aims/Hypothesis: To examine the hypothesis that connexin hemichannel blockade after global cerebral ischemia improves survival of striatal GABAergic neurons.

Methods: A mimetic peptide that blocks connexin 43 hemichannels was infused into the lateral ventricle of chronically instrumented fetal sheep *in utero* at 128 ± 1 days (0.87) of gestation. Short (1 h, n = 5) or long (25 h, n = 6) infusion of peptide or vehicle (n = 6) was started 90 minutes after 30 minutes of severe cerebral ischaemia induced by reversible bilateral carotid artery occlusion. Fetal electroencephalography (EEG) was continuously monitored before, during and for 7 days after cerebral ischemia. At post mortem, fetal brains were collected for immunohistochemical assessment of striatal GABAergic neurons, including: calbindin-28k, calretinin, parvalbumin and glutamic acid decarboxylase (GAD) positive neurons.

Results: Cerebral ischaemia was associated with loss of calbindin 28k, calretinin, parvalbumin and GAD positive neurons ($P<0.05$ vs. sham ischaemia). Short infusion of peptide did not improve survival of any striatal phenotype compared to ischaemia+vehicle. Long infusion of peptide was associated with increased survival of calbindin-28k, calretinin and parvalbumin positive neurons ($P<0.05$ vs. ischaemia+vehicle), but did not significantly improve survival of GAD positive neurons ($P=0.08$ vs. ischaemia+vehicle). Improved survival of calbindin-28k, calretinin and parvalbumin positive neurons was strongly associated with reduced seizure burden and improved recovery of EEG at 7 days after hypoxia ischaemia ($P<0.05$, ischaemia+long infusion vs. ischaemia+vehicle).

Conclusions: Connexin hemichannel blockade after fetal cerebral hypoxia-ischaemia improved survival of striatal GABAergic neurons and was strongly associated with reduced seizure burden and improved recovery of brain activity. Collectively, these data suggest that blockade of connexin hemichannels after asphyxia has the potential to reduce basal ganglia injury.

Non-additive neuroprotection with delayed cerebral hypothermia and recombinant human erythropoietin infusion after prolonged cerebral ischemia in near-term fetal sheep

Guido Wassink, Joanne O. Davidson, Caroline A. Yuill, Mhoyra Fraser, Laura Bennet, and Alistair J. Gunn

Fetal Physiology and Neuroscience Group, Dept of Physiology, University of Auckland, Auckland, New Zealand.
aj.gunn@auckland.ac.nz

Background: Current protocols for hypothermia treatment for hypoxic-ischemic encephalopathy are incompletely neuroprotective. The pleiotropic growth factor, recombinant human erythropoietin (rh-EPO) has shown promising neuroprotective effects in neonatal animals, but there is limited large animal evidence for benefit when it is combined with hypothermia.

Aims/Hypothesis: To determine whether combined treatment with delayed cerebral hypothermia plus rh-EPO can further improve outcomes.

Methods: Term equivalent fetal sheep (0.8 gestation) received 30 min of global cerebral ischemia. From 3 to 72 hours after ischemia fetuses received either normothermia + vehicle infusion (ischemia-control, n=8), or cerebral hypothermia (ischemia-hypo, n=8), or continuous rh-EPO infusion (ischemia-EPO, 5000 IU/kg loading dose, then 5000 IU/kg every 6 hours, n=8), or combination treatment with cerebral hypothermia plus rh-EPO (ischemia-EPO-hypo, n=8). Post-mortem was performed 7 days after cerebral ischemia.

Results: Cerebral ischemia was associated with marked neuronal loss and induction of microglia in the parasagittal cortex. Hypothermia was associated with reduced neuronal loss ($p<0.001$) and microglial induction ($p<0.01$) in the parasagittal cortex, with greater overall recovery of EEG power and spectral edge frequency from 48 hours onwards ($p<0.001$). Ischemia-EPO was associated with improved neuronal survival ($p<0.05$), reduced induction of Iba1-positive microglia ($p<0.001$), and faster recovery of spectral edge frequency but not EEG power compared to ischemia-control ($p<0.05$) from 120 hours onwards. Ischemia-EPO-hypo was not significantly different from ischemia-hypo for any outcome.

Conclusions: These preliminary findings suggest that delayed hypothermia and recombinant human erythropoietin are independently neuroprotective, but delayed induction of combined hypothermia and rh-EPO after cerebral ischemia was not associated with additive neuroprotection in near-term fetal sheep. We speculate that this reflects overlap in the pathways targeted by hypothermia and by EPO.

Hypoxia-induced neonatal seizures are independently associated with reduced $GABA_A$ α_3 subunit expression

Stephanie M Miller, Susan M Sullivan, Zoe Ireland, Kirat K Chand, Paul B Colditz, S. Tracey Björkman

The Perinatal Research Centre, UQ Centre for Clinical Research, The University of Queensland, Herston, Australia.
s.odriscoll@uq.edu.au

Background: Seizures are a common manifestation of hypoxic-ischaemic brain injury in the neonate. In status epilepticus models alterations to $GABA_AR$ subunit expression have been suggested to contribute to 1) abnormal development of the GABAergic system, 2) why seizures become self-sustaining and 3) the development of pharmacoresistance. Detailed investigation of $GABA_AR$ subunit protein expression after neonatal hypoxia-ischaemia (HI) is currently insufficient.

Aims/Hypothesis: Using our pig model of HI and subsequent spontaneous neonatal seizures, we investigated changes in protein expression of the three predominant α-subunits of the $GABA_AR$; α_1, α_2 and α_3. It was hypothesised that neonatal HI-induced seizures would result in changes to α-subunit protein expression.

Methods: Anaesthetised and ventilated newborn pigs (<24h old, n=47) were subjected to 30 min hypoxia (4% iO_2) including 10min systemic hypotension and recovered to 24 or 72h. Control animals (n=13) underwent the same surgical procedures with no HI. Amplitude-integrated electroencephalography was used to monitor brain activity and identify electrographic seizure activity. Brain tissue was collected post mortem and $GABA_AR$ α-subunit protein expression was analysed using western blot and immunohistochemistry. Correlation and regression analyses were used to investigate the relationship between HI-induced seizures and $GABA_AR$ α-subunit changes.

Results: $GABA_AR$ α_1 and α_3 protein expression was significantly reduced in animals that developed seizures after HI particularly at 72h; HI animals that did not develop seizures did not exhibit the same reductions. Immunohistochemistry revealed decreased α_1 and α_3 expression, and redistribution of the α_1 subunit from the cell membrane to the cytosol, in the hippocampus of seizure animals. Quantification of α_3-positive cells, normalised to NeuN immunoreactivity, showed a significant decrease in α_3 labelling in the animals exhibiting seizures compared with HI animals and controls, independent of HI-induced cell death and this was confirmed with regression analysis.

Conclusions: This is the first study to show loss and redistribution of $GABA_AR$ α-subunits in a neonatal brain experiencing seizures. Our findings are similar to those reported in models of SE and in chronic epilepsy.

A proposed study for investigation of the blood-brain barrier in neonatal seizures and hypoxic ischaemic encephalopathy

Kate Goasdoue, Stephanie Miller, Kirat Chand, Julie Wixey, Tracey Björkman

Perinatal Research Centre, Centre for Clinical Research, University of Queensland, Brisbane, Australia.
Kate.goasdoue@uqconnect.edu.au

Background: The blood-brain barrier (BBB) is an integral part of a functioning CNS. A fully functional and intact BBB maintains the homeostasis of metabolites, ions, and water and protects the brain from neurotoxic substances. Disruption of the BBB is involved in a number of CNS disorders, including seizure. BBB disruption has been investigated in adult and animal models but it is not well understood or characterised in the neonate. Up to 60% of neonatal seizures are associated with hypoxic-ischaemic encephalopathy – a disorder of which BBB disruption is also a hallmark. Disruption of the BBB has been shown in adults to worsen and alter the progression of seizures; additionally, seizure has been demonstrated to induce and exacerbate BBB disruption. Increasing our understanding of the mechanisms involved in seizure generation and progression in the neonate is not only vital because neonatal seizures often do not respond to treatment with anti-epileptic drugs but because neonatal seizures are often symptomatic of CNS pathology. Investigating the BBB in these conditions is a major untapped area of research and potential avenue for novel treatments.

Aims/Hypothesis: The aim of the proposed study is to measure the degree and timing of BBB disruption in a clinically relevant model of hypoxic ischaemic encephalopathy and consequent seizures. It is hypothesised that BBB disruption will be greatest in animals with higher levels of hypoxic ischaemic injury and seizure burden. It is also hypothesised that earlier BBB disruption will predict earlier onset of seizure.

Methods: A neonatal piglet model of hypoxia-ischaemia with spontaneous seizures will be utilised. Blood biomarkers will be analysed to test the degree and time-course of blood brain barrier disruption in this model. BBB disruption will also be analysed with qPCR and IF markers. The results will be compared with outcome measures of hypoxia ischaemia (neuropathology scores, MRI/MRS data, TUNEL) and seizure burden analysis to determine the relationship between BBB disruption and outcome.

Results: As this is a proposed study there are no results to report at this stage.

Impact: Analysis of the BBB is a severely lacking area of research that could potentially inform treatments for neonatal seizures and CNS pathologies in addition to increasing our understanding of the underlying mechanisms.

Expression of inflammatory mediators after hypoxic-ischemic brain injury in a neonatal pig model

Kirat Chand, Kate Goasdoue, Stephanie Miller, Paul Colditz, Tracey Björkman

Perinatal Research Centre, University of Queensland Centre for Clinical Research, The University of Queensland, St. Lucia, 4067, Australia.
k.chand@uq.edu.au

Background: Perinatal hypoxic/ischemic (HI) brain injury is a common cause of neurodevelopmental outcomes such as cerebral palsy. A number of processes are initiated following HI, of particular interest to the present study is the expression of pro- and anti-inflammatory mediators which play a significant role in the severity of brain injury. These mediators also influence blood brain barrier (BBB) disruption making the brain more susceptible to injury. While the influence of these cytokines and chemokines in progression of HI brain injury is well studied, there is minimal research into their role in neonatal brain injury following BBB disruption. Understanding the time course of these mediators may aid in advancement of treatments to improve neurodevelopmental outcomes of neonates after HI injury.

Aims/Hypothesis: To investigate the time course in expression of pro- and anti-inflammatory mediators following HI brain injury in a neonate pig model.

Methods: Newborn piglets (<24 h after birth) were used for this study. Following stable physiological variables for one hour, hypoxia was induced by decreasing FiO_2 to 4% and adjusted to achieve aEEG peak amplitude <5 μV and hypotension <30 mmHg for the final 10 minutes of a 30 minute HI period. Sham animals underwent the same procedures except for HI insult. Piglets were euthanized 1, 4 or 24 hours after commencement of treatment and brain tissue from parietal cortex was collected post-HI. Brain injury was assessed using early markers of brain injury, including gene expression of inflammatory markers and immunofluorescent labelling of markers. mRNA expression of markers was examined using pig specific RT^2 Profiler PCR Arrays of inflammatory mediators. IHC analysis included examination of degenerating neurons, microglia and astrocytes using Fluoro-Jade C, Iba1 and GFAP respectively.

Results: Preliminary results show early upregulation of pro- and anti-inflammatory markers following one hour HI, with a significant increase in the pro-inflammatory markers CCR5, IL5 and VEGF in HI brains compared with sham. Pro-inflammatory markers were further elevated by four hours with a down regulation of anti-inflammatory markers. Morphological examination showed increased expression of astrocytes and degenerating neurons in HI brains.

Conclusions: Our early data indicate HI injury results in rapid upregulation of pro-inflammatory markers in the neonate brain. Our future studies will extend the current preliminary work and also examine whether current therapies such as hypothermia alter the expression levels of these mediators in the neonate HI injured brain.

Understanding the association between seizures and neuronal degeneration following birth asphyxia, and the neuroprotective potential of umbilical cord blood stem cells

Riana Samuel, Tamara Yawno, Courtney McDonald, Graham Jenkin, Suzanne Miller

Hudson Institute of Medical Research, 27 - 31 Wright Street, Clayton VIC 3168, The Ritchie Centre, Monash University, Melbourne, Australia.
rsam16@student.monash.edu

Background: Birth asphyxia, resulting from a severe reduction in oxygen supply to the infant during labour and/or birth, is a significant cause of infant death or long-term neurodevelopmental disability. Hypoxic-ischaemic encephalopathy (HIE), the clinical syndrome arising from birth asphyxia, is strongly associated with newborn seizures and long-term poor outcomes for the brain, including cerebral palsy.

Currently there is great interest in clinical trials for the use of umbilical cord blood (UCB) stem cells for infants with HIE, however their mode of action is not understood and it is not known if they reduce seizure activity within the brain after asphyxia. Seizures themselves are linked to neurodegeneration after an asphyxic insult.

Aims: This study aimed to understand the relationship between seizures and neuronal cell death in the brain after birth asphyxia, and whether administration of UCB stem cells alters seizure activity and cell death.

Methods: Near-term fetal sheep were used in the study, corresponding to the neuronal development of term human infants. We used an established model of birth asphyxia in lambs to impose HIE, and lambs were maintained after birth for 48 hours under neonatal intensive care conditions, with continuous electroencephalogram (EEG) monitoring of brain activity. Autologous UCB mononuclear cells (~100 million) were administered IV to a subset of lambs at 12 hours following asphyxia. At 48 hours after asphyxia, lambs were euthanised and underwent a post mortem for brain collection.

Results: Lambs were divided into 3 groups: control (n=3), birth asphyxia (asphyxia; n=4) and asphyxia + UCB stem cells (asphyxia + UCB; n=4). All asphyxia and asphyxia + UCB lambs had electrographic seizures, with seizures commencing before 12 hours after birth asphyxia in 5 of 9 asphyxia lambs. Brain analysis is continuing and results to date demonstrate that UCB stem cells do not reduce the presence of seizures but decrease neuronal cell death (apoptosis).

Conclusion: UCB stem cells did not prevent the onset of seizures; in most cases seizure activity had commenced prior to cell administration at 12h after birth. We did however observe a trend towards an improvement in neuronal survival and brain architecture with UCB administration. Combined, this suggests that timing of UCB stem cells may be critical after birth asphyxia.

Session 3

Chairs – Nicolette Hodyl and Leo Leader			
2:15	**A13**	Rosemary Horne	Sleeping like a baby – is this really a good thing?
2:35	**A14**	Dawn Elder	24 – hour oxygen saturation recordings at discharge in preterm infants – prevalence of intermittent hypoxia and comparison of different methods of editing data
2:47	**A15**	Helena Parkington	Potassium channel dysfunction in failed human labour
2:59	**A16**	Nadia Bellofiore (E)	Pre-menstrual Mouse? The common spiny mouse as a model for PMS
3:11	***A17**	Jane Pillow	Antenatal inflammation ablates early postnatal development of circadian rhythm in preterm fetal lambs
3:18	General discussion		

Sleeping like a baby – is this really a good thing?

Rosemary SC Horne

The Ritchie Centre, Hudson Institute of Medical Research and Department of Paediatrics, Monash University, Melbourne, Australia.
rosemary.horne@monash.edu

Sleep is vital for sustaining infant brain development. Term infants spend up to 70% of each 24 hours asleep, while preterm infants devote almost 90% of their day to sleeping. It is well established that sleeping position is an important part of a safe sleeping environment for the term infant. Prone sleeping significantly increases the risk of the Sudden Infant Death Syndrome (SIDS). The recommendation that all infants are slept in the supine position has reduced SIDS by over 80% worldwide. In healthy term infants, we have previously shown that the increased risk of SIDS with prone sleeping is associated with a range of adverse effects imposed by this position on infant physiology, including reduced cerebral oxygenation and blood pressure, impaired autonomic cardiovascular control and altered neural function (as evidenced by reduced arousability from sleep).

Around 10% of all infants are born preterm. Preterm birth is a global problem and the annual total number of preterm births in 65 countries (including Australia and New Zealand) has increased by 0.2 million over the last 20 years. Ex-preterm infants are at four times increased risk of SIDS. It is the usual practice for preterm infants in a hospital neonatal intensive care unit (NICU) or special care nursery to be slept prone for ≥50% of the time. This is because it is commonly believed that prone sleeping improves respiratory function. However, studies have shown conflicting results as to whether prone sleeping in preterm infants improves respiratory function in the short-term.

We have recently studied ex-preterm infants longitudinally after discharge home and found more pronounced adverse effects of prone sleeping on cerebral oxygenation, cardiovascular and cerebrovascular control in these babies than in term-born infants across the first 6 months of post-term corrected age. These findings likely underpin the increased risk of SIDS in ex-preterm infants

It is essential that parents receive safe sleeping messages for their preterm infants and that safe sleeping is practiced whilst in the neonatal unit as soon as medically possible so that parents can model this when they come home.

24 – hour oxygen saturation recordings at discharge in preterm infants – prevalence of intermittent hypoxia and comparison of different methods of editing data

Grace Wellington[1], Angela Campbell[2], Dawn Elder[1]

Departments of Paediatrics & Child Health[1] and Medicine[2], University of Otago, Wellington, NZ.
dawn.elder@otago.ac.nz

Background: There is limited literature documenting intermittent hypoxia (IH) at preterm infant discharge using new generation oximeters and none addressing artefact in these recordings.

Aims: The aims of this study were to: 1. Determine the prevalence of IH in preterm infants at neonatal discharge by documenting 3%DSI and 4%DSI from a 24 hour oximetry recording. 2. To document differences between automatically edited, manually edited and unedited oximetry data. 3. Compare overnight 12-hour recordings to full 24-hour recordings.

Methods: Infants < 37 w gestational age (GA) admitted to Wellington NICU were recruited. Masimo Rad – 8 oximeters were used with a 2 second averaging time. 24-hour oximetry recordings were performed close to discharge. Three editing modes were compared: Manual, Automatic (profox software) and no editing. For infants < 32 w GA recordings were repeated 1 month post discharge.

Results: 38 infants (17 males) had a 24-hour pulse oximetry recording suitable for analysis. Median GA at birth was 32.5 weeks (range 24-36). Postmenstrual age at time of study varied from 35 to 42 completed weeks. Auto-edited reports gave similar results to manually edited reports so these data are presented. Median mean SpO2 was 97.9 (97.2-98.8), median DSI3% 77.4 events/hour (52.5-103.1) and median DSI4% 51 events/hr (31.1-73.7). For 12 infants who had a repeat study the median DSI4% decreased from 57.9 events/hr to 25.5 events/hr (p=0.008). 24-hour oximetry reports were clinically similar to 12-hour recordings.

Conclusion: The prevalence of IH is high in preterm infants at neonatal discharge but decreases in very preterm infants four weeks post discharge. Automatically editing oximetry reports gives similar results to manual editing for most clinically important measures.

Potassium channel dysfunction in failed human labour

Helena C Parkington[1], Mary A Tonta[1], Janet Stevenson[2], Crystal Goundar, Penelope J Sheehan[2], Harold A Coleman[1], Shaun P Brennecke[2]

[1]Department of Physiology, Monash University, Melbourne, Australia, [2]Department of Perinatal Medicine Royal Women's Hospital, University of Melbourne, Melbourne, Australia.
helena.parkington@monash.edu

Background: Strong labour contractions require Ca^{2+} influx through voltage-gated Ca^{2+} channels. Thus, myometrial smooth muscle membrane potential (voltage difference) is critical for good labour progress. Failure to progress in labour (FTP) is a significant problem in the labour ward, and is the most common indication for caesarean delivery (CD). The incidence of CD has doubled in recent years, strongly linked to the rise in obesity. In a recent study we discovered a marked excessive negative membrane potential in myometrium of both lean and obese women undergoing CD for FTP.

Aims/Hypothesis: Our hypothesis was that this negative membrane potential was caused by excessive activity and/or levels of K^+ channels, the resulting negativity suppressed the opening of Ca^{2+} channels and results in weak contraction. Likely K^+ channel candidates identified in human myometrium include K_V2.1, 3.4, 4.1, 4.3, 7.1, 7.4, 11.1 and K_{IR}7.1.

Methods: Electrophysiology was used to record membrane potential and K^+ channel activity in term not-in-labour (NIL) and in labour (IL) myometrium. K^+ channel protein levels were determined using western blotting.

Results: Myometrial strips from women progressing well IL had spontaneous contractions (n=7). FTP strips did not contract spontaneously (n=9), although contraction could be achieved using K^+ rich solution to evoke large depolarization. Resting membrane potential in myometrium from normally progressing women before labour was -58 ± 1 mV (n=17) and IL was -58 ± 1 mV (n=7). FTP myometrium was significantly more negative IL (-73 ± 2 mV, n=9). Of the likely K^+ channels responsible, blockade of K_V7.1 and 7.4 alone, using XE-991, returned the resting potential to normal levels (-61 ± 2 mV) in high negative FTP myometrium. Levels of K_V7.1 protein did not change in myometrium from normally progressing women before versus in labour, but was significantly increased in FTP (21.2 ± 2.4, n=5) versus normal progress IL (11.9 ± 1.6, n=5, p=0.02). Levels of K_V7.4 protein were also significantly increased in myometrium from FTP IL women (1.18 ± 0.14, n=5) versus normally progressing labour (0.26 ± 0.04, n=5, p=0.0008). In acutely isolated myometrial cells the K_V7.1/4 current (at 20mV) was enhanced in IL FTP (6.1 ± 1.1pA/pF) versus normal progress (2.5 ± 0.5pA/pF, p=0.02). In FTP myometrium, the depolarization evoked by oxytocin (10nM) was 8 ± 2mV, insufficient to overcome the negativity and so did not cause contraction.

Conclusions: Dysfunction of K_V7.1/4 channels is a major contributor to FTP in human labour.

Pre-menstrual Mouse? The common spiny mouse as a model for PMS

Nadia Bellofiore[1,2], Stacey Ellery[1,2], David Walker[1,2], Peter Temple-Smith[2] and Hayley Dickinson[1,2]

[1]The Ritchie Centre, Hudson Institute of Medical Research, 27-31 Wright St, Clayton, Australia 3168; [2]Obstetric and Gynaecology, Monash University, Clayton, Australia 3168.
Nadia.bellofiore@hudson.org.au

Background: Pre-menstrual syndrome (PMS) is a well-recognised affliction in modern society, with up to 90% of women experiencing symptoms forewarning of imminent menstruation. These include physiological changes such as bloating, breast pain or abdominal cramping and are commonly combined with emotional distress. The severity and subtypes of PMS are subject to individual variation, but studies suggest that around half of these women's symptoms are extreme enough to seek intervention. Due to a lack of an appropriate animal model, the mechanisms of PMS are not yet well-understood, and consequentially, efficacious treatments and effective relief for women is limited.

Aims/Hypothesis: Our laboratory has recently discovered the first naturally-occurring menstruating rodent, the common spiny mouse (Acomys cahirinus); in which the events of spontaneous decidualisation, progesterone withdrawal, endometrial shedding and vaginal bleeding closely mimic that of the human menstrual cycle. Hence, we are seeking to determine whether they also exhibit notable behavioural changes in correlation with their menstrual cycle stage. We hypothesise that spiny mice experience an analogous pre-menstrual state.

Methods: Virgin females (6-12 months of age) are subjected to daily vaginal lavage for 10 consecutive days. Qualitative behavioural responses to researcher handling (e.g. ease of capture and restraint, frequency of vocalisations, response to abdominal palpation, etc.) is recorded on an increasing scale of normal to severe. Females are isolated in a metabolic cage for a period of 24h to assess food and water intake across the cycle.

Results: Of the n=13 spiny mice currently assessed, we found no statistically significant correlations between stage of the menstrual and behavioural response to researcher handling. However, in ten of eleven parameters tested, the odds of a female responding severely (e.g. severe response to abdominal palpation where $p \sim 0.2$) is approximately twice as high if a female is within 24h of menstruation compared to early follicular phase females. Metabolic cage analysis show a trend of increased consumption of food and water and weight gain in the 24h preceding menstrual bleeding.

Conclusions: Patterns observed in spiny mouse behaviour suggests some degree of correlation with stage of the menstrual cycle. These studies are ongoing and will be extended to quantify behaviour, using common rodent behavioural tests (Open Field, Elevated Plus Maze and Social Interaction tests), already validated in the spiny mouse. Urinary cortisol and catecholamine metabolites will be also assayed to determine peaks periods of stress and anxiety.

Antenatal inflammation ablates early postnatal development of circadian rhythm in preterm fetal lambs

Whittle RA[1,2], Pillow JJ[1,2], Noble PB[1,2], Ahmadi-Noorbakhsh S[1,2], Mark P[1]

[1]Anatomy, Physiology & Human Biology; & 2Centre for Neonatal Research & Education, Univ. of Western Australia, Perth.
jane.pillow@uwa.edu.au

Background: Circadian rhythmicity is central to normal physiological function in multiple organ systems. Circadian rhythms exist in the fetus via maternal signals. Postnatally, circadian rhythm maintenance is dependent on successful synchronisation of the biological clock with external environmental cues. Preterm infants are subjected to abnormal environments devoid of normal circadian cues. Additionally, many preterm infants are exposed antenatally to chorioamnionitis, a proinflammatory stimulus that disturbs neurological function.

Aims: 1) To determine the effects of preterm delivery on postnatal development of circadian rhythm in salivary cortisol secretion; and 2) To identify any additional impost of an antenatal pro-inflammatory exposure on the postnatal circadian rhythm of salivary cortisol secretion.

Methods: Fourteen pregnant ewes received intra-amniotic saline (2 mL, n=8) or lipopolysaccharide (LPS from E.coli 055:B5; 4 mg/2 mL, n=6) at 126 d gestation (term ~150 d). Preterm lambs were exteriorised, intubated and delivered by caesarean section, prior to resuscitation, ventilation and ongoing postnatal management in the preclinical intensive care research unit under continuous lighting conditions. Term lambs (n=3) were delivered naturally and reared with their mothers in light/dark cycle. Saliva was collected on day 7 (Salivette ®, Sarstedt AG & Co, Germany), centrifuged (4100 rpm, 2.14 g, 15 min, 40C), then snap frozen at -800C. Cortisol was assayed using a salivary enzyme immunoassay kit (Salimetrics ®, Carlsbad, USA). All measurements were made by a single assessor (RW) blinded to the treatment group.

Results: Term lambs exhibited a significant circadian rhythm (Mean (SEM): Mesor , 0.202 (0.009) µg/dL; Amplitude 0.119 (0.013) µg/dL; Delay 5.13 h past midnight, p=0.0002). Saline exposed preterm lambs exhibited a trend towards establishment of a circadian rhythm (p=0.086), however the phase was delayed compared to term lambs. LPS lambs exhibited a flat salivary cortisol profile with no evidence of circadian rhythmicity (p=0.824).

Conclusions: Term lambs delivered and reared by their mother in an indoor environment with 12 hour artificial light cycling establish circadian rhythmicity by day 7. The combination of premature birth and management within a constantly lit intensive care environment likely delays establishment of circadian rhythmicity and disrupts normal phasic alignment. Ablation of circadian rhythmicity by an antenatal pro-inflammatory stimulus is a novel finding that may reflect a more generalised adverse impact of inflammation on the developing brain and warrants further investigation.

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Session 4

Chairs – Michael Stark and Jon Hirst			
9:15	**A18**	Abby Fowden	Glucocorticoid programming of intrauterine development
9:45	**A19**	Jack Darby (E)	Preterm birth coupled with antenatal glucocorticoid treatment increases cardiac MR and 11β-HSD1 in adult life
9:57	**A20**	Ishmael Inocencio (E)	Nitric oxide as a potential therapeutic against cardiovascular dysfunction in fetal growth restricted fetuses
10:09	**A21**	Tim Cole	Glucocorticoids regulate cell type-specific pathways in the interstitial mesenchyme and epithelial compartments of the mammalian fetal lung
10:21	**A22**	Jia Yin Soo	The effect of substrate supply on fetal hepatic gene expression of drug transporters and drug metabolising enzymes
10:33	***A23**	Tamás Zakár	Epigenetic regulation of CRH expression in human trophoblasts
10:40	***A24**	Emma Buckels (L)	Developing a method to visualise the endocrine pancreas using whole-slide imaging
10:47	General discussion		

Glucocorticoid programming of intrauterine development

AL Fowden, OR Vaughan, JK Jellyman, OA Valenzuela & AJ Forhead

Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, CB2 3EG, UK.
alf1000@cam.ac.uk

In adults, glucocorticoids (GCs) are stress hormones that aid survival during challenges to homeostasis. In fetuses, they have an even wider range of roles. Towards term, GCs act as the maturational signal in preparing fetuses for birth. Earlier in gestation, raised GC concentrations in either the fetus or mother can act as environmental signals that alter fetal development in relation to resource availability for intrauterine growth. These functions improve viability before and at birth, particularly when conditions are sub-optimal for survival. However, by altering fetal growth, early exposure to excess GCs modifies the developing phenotype with life-long consequences. Consequently, GCs also act as programming signals that adapt intrauterine development to optimise offspring viability and fitness.

Both before and at term, GCs affect development of a wide range of fetal tissues by inducing changes in cellular expression of structural, transport and signalling proteins, which have widespread functional consequences at whole organ and system levels. Glucocorticoids, therefore, activate many of the physiological systems that have little or no function *in utero* but are vital at birth, such as pulmonary respiration, hepatic glucogenesis and themoregulation. At the tissue level, their developmental effects can be direct via glucocorticoid receptors or mediated indirectly via changes in the placenta or other endocrine systems. At the molecular level, GCs can act directly on gene expression via the promotors or indirectly by epigenetic modifications to the genome. However, in switching tissues to differentiation from accretion to improve immediate viability, GCs can lead to long term functional deficits, particularly if excess exposure occurs before full term or affects tissues like the placenta that can alter fetal development long after the original insult. For instance in pregnant mice and sheep, raising maternal GC concentrations in late pregnancy alters placental transport and metabolism of glucose with consequences for fetal metabolism and growth. In horses in which fetal HPA development occurs comparatively late in gestation, the main period of susceptibility to glucocorticoid programming may be immediately after rather than before birth. Certainly, raising cortisol concentrations in newborn foals for 5 days leads to altered insulin secretion in older foals and to abnormal insulin sensitivity and HPA function in yearlings. Thus, GCs are important regulatory signals during both fetal and early neonatal life. They program a phenotype best suited to the prevailing environmental conditions, thereby maximising the chances of offspring survival to reproductive age. However, if postnatal conditions differ from those signalled *in utero*, the glucocorticoid-induced developmental adaptations can become maladaptive with adverse outcomes for offspring health in the long term.

Preterm birth coupled with antenatal glucocorticoid treatment increases cardiac MR and 11β-HSD1 in adult life

Jack R Darby[1], Max Berry[2,3*], Rebecca Dyson[2], Clint Gray[2] and Janna L Morrison[1*]

*[1]Early Origins of Adult Health Research Group, School of Pharmacy & Medical Sciences, Sansom Institute for Health Research, University of South Australia, Adelaide, SA, Australia. [2] Centre for Translational Physiology, University of Otago, Wellington, New Zealand. [3] Department of Paediatrics and Child Health, University of Otago, Wellington, New Zealand. *=equal contribution*
jack.darby@mymail.unisa.edu.au

Background: It is estimated that 15 million babies are born preterm globally each year. Although the link between preterm birth and short term morbidity is relatively clear; the long term consequences of preterm birth on adult health are not completely understood. Indeed, there is evidence to suggest that preterm adult offspring may have an increased risk of developing of cardio-metabolic diseases. Herein, we developed a guinea pig model of medically induced preterm birth, designed to be more closely related to the human preterm pregnancy by preserving maternal-infant bonding and antenatal corticosteroid exposure.

Aim: The present study aimed to use a closely comparable model of the human preterm pregnancy to investigate potential effects on cardiac signalling pathways and if these effects persisted from childhood to adulthood.

Methods: Betamethasone was administered to pregnant guinea pigs 48 and 24 h before induction of preterm labour (62d gestation) in order to mimic the standard care for women at risk of preterm delivery. Cardiac tissue was collected at 28d and 9 months. qRT-PCR was used to determine the mRNA expression of molecules involved in the regulation of cardiac development.

Results: Preterm birth with exposure to antenatal glucocorticoids did not alter the mRNA expression of the glucocorticoid receptor (GR) or 11β-HSD2, which converts cortisone to cortisol. However, at 9 months of age pups that were born preterm had an increase in the mRNA expression of the mineralocorticoid receptor (MR) and 11β-HSD1, the cortisol to cortisone converting enzyme. Furthermore, these changes were consistent across both sexes.

Conclusions: In this study, we have used an animal model that is closely comparable to human preterm pregnancy to show that those offspring born preterm have altered mRNA expression of MR and 11β-HSD1. MR signalling has previously been implicated in cardiac hypertrophy independent of increased blood pressure, as such the finding that preterm adult offspring have increased cardiac mRNA expression of MR may shed some light on the molecular pathways involved in the cardiac changes that increase the risk of preterm adults developing cardio-metabolic disease.

Nitric oxide as a potential therapeutic against cardiovascular dysfunction in fetal growth restricted fetuses

Inocencio IM, Polglase GR, Sutherland A, Mihelakis J, Miller S, Allison BJ

The Ritchie Centre, Hudson Institute of Medical Research and Department of Obstetrics and Gynaecology, Monash University, Melbourne, Australia.
ishmael.inocencio@monash.edu

Background: Fetal growth restriction (FGR) commonly occurs due to inadequate delivery of nutrients to the fetus secondary to placental impairment. Currently there is no therapy for FGR. Experimental evidence has demonstrated that FGR fetuses redistribute their cardiac output *in utero* to survive persisting suboptimal conditions (e.g. chronic hypoxia). We have previously shown that ventilation with inhaled nitric oxide (NO) increases left ventricular output (LVO) and cerebral blood flow (CBF) in FGR fetuses (Polglase et al. *in press*), indicating improved cardiac output. These results suggest an altered NO pathway may underlie the pathophysiology of FGR infants.

Aims/Hypothesis: The aim of the current study is to further investigate and characterize the mechanisms by which NO provides therapeutic benefit against cardiovascular dysfunction in FGR infants. Specifically, we will compare *in vitro* vascular function and vascular morphology focussing on NO pathways of FGR and AG fetuses in critical vascular beds; cerebral, pulmonary, systemic and peripheral resistance vessels.

Methods: Preterm lambs (0.6 gestation) underwent sterile surgery to induce FGR by single umbilical artery ligation (SUAL) or sham surgery (control, AG). At 0.83 gestation lambs were delivered and immediately euthanized for collection of proximal and distal regions of aorta, carotid, femoral, pulmonary and middle cerebral artery vessels. Proximal sections were used for assessment of vessel function via *in vitro* wire myography and mediators of the NO pathway were interrogated. Distal sections were both immediately frozen and fixed for structural and molecular analysis.

Results: *In vitro,* sildenafil administration resulted in a significant ($p<0.05$) increase in vasodilation (max relaxation, as a % of total contraction) within the peripheral, systemic and cerebral vasculature of FGR compared to AG offspring. Cerebral vessels also demonstrated significantly increased sensitivity (EC50, $p<0.05$) to sildenafil in FGR offspring. No difference in vasodilation was observed in pulmonary arteries. Histological and molecular analysis of vessel structure is currently underway.

Conclusions: The increased vasoreactivity of FGR vessels to sildenafil suggests that FGR offspring upregulate downstream targets of the NO pathway that may be able to utilised for therapeutic benefit. Further studies are justified to improve cardiovascular outcomes for FGR offspring.

Glucocorticoids regulate cell type-specific pathways in the interstitial mesenchyme and epithelial compartments of the mammalian fetal lung

Cole TJ[1], Seow B[1], Short K[1] and Ng J[1], Hooper SB[2], Bird AD[1, 3]

[1]Department of Biochemistry and Molecular Biology, Monash University, Melbourne, Victoria, 3800, Australia; [2]The Richie Centre & [3]Hudson Institute, Monash Medical Centre, Melbourne, Victoria, 3168, Australia.
tim.cole@monash.edu

Background: Organogenesis in the developing mammalian embryo proceeds via an integrated program of cell intrinsic determination, cell proliferation and terminal differentiation, in part via response to coordinated local and systemic hormonal cell signalling. The adrenal steroid cortisol activates the intracellular glucocorticoid nuclear receptor (GR) and is an important maturation hormone during the final stages of lung development to complete respiratory development allowing the transition to respiration at birth. Synthetic antenatal glucocorticoids are used to accelerate fetal lung maturation in preterm birth yet how these steroids work mechanistically at the cellular level in the lung is not fully understood, as are the potential detrimental side-effects of antenatal and postnatal synthetic steroids in other developing organs.

Aims/Hypothesis To investigate the specific cellular roles of cortisol in the respiratory system of the developing fetus using genetic mouse models, where cortisol cell-signalling has been ablated via cell-type specific targeted mutation of the glucocorticoid receptor (GR) gene.

Methods: We ablated GR-mediated glucocorticoid signalling in the mesenchyme, epithelial cell and endothelial cell compartments of the developing mouse respiratory system using Cre-loxP gene-targeting techniques. Mice were analysed for perinatal survival, lung histology/morphology, and changes in gene expression profiles using transcriptome sequencing.

Results: Global deletion of the GR in the fetal lung causes perinatal death with major deficits in lung development. Mesenchyme-specific deletion of the GR recapitulates this phenotype and defines a critical lung compartment for glucocorticoid action. Surprisingly lung epithelial cell-specific deletion of GR signalling still allows normal lung function and survival at birth, although any postnatal deficits of lung function and compliance have not been determined. Whole tissue and primary cell NGS RNA-sequencing analysis in GR-mesenchyme and GR-lung epithelial cell targeted mouse models has profiled specific subsets of GR-regulated target genes and downstream gene networks. These include mesenchymal regulation of ECM genes such as versican, tropoelastin, and fibrillin 2, and epithelial cell regulation of surfactant metabolism, cell differentiation and growth factors.

Conclusions: These results show a critical role for glucocorticoid signalling in the lung mesenchyme for normal lung maturation and begins to define steroid regulated cell-type specific signalling networks in the developing lung.

The effect of substrate supply on fetal hepatic gene expression of drug transporters and drug metabolising enzymes

Jia Yin Soo[1], I Caroline McMillen[1] and Janna L Morrison[1]

[1]Early Origins of Adult Health Research Group, School of Pharmacy & Medical Sciences, Sansom Institute for Health Research, University of South Australia, Adelaide, SA, Australia.
jia_yin.soo@mymail.unisa.edu.au

Background: Drugs are often required to treat illness during pregnancy in order to obtain the best outcomes for both mother and fetus. Evidence from human and animal studies show that reduced or excess substrate supply to the fetus is accompanied by hormonal and metabolic changes, particularly in hormones such as glucocorticoids and insulin. These hormonal and metabolic changes may effect fetal hepatic expression of drug transporters and metabolising enzymes and hence may alter fetal drug exposure.

Aims/Hypothesis: This study aims to determine the effect of reduced or excess substrate supply to the fetus on the expression of drug transporters and metabolising enzymes in the liver before and after birth.

Methods: In the reduced substrate cohort, placental restriction (PR) leading to growth restriction was induced in ewes using carunclectomy. Livers were collected from the fetuses at 140d gestation (Control, n=8; PR, n=7) and lambs 21d after birth (Control, n=8; PR, n=8). In the excess substrate cohort, ewes were randomized to either late gestation control (100% of metabolisable energy requirement (MER)) or overnutrition (LGON; 170-190% MER) group from 115d. Livers were collected from the fetuses at 140d gestation (Control, n=5; LGON, n=8) and lambs 30d after birth (Control, n=10, LGON, n=8). mRNA abundance of genes that regulate the expression of drug transporters and metabolising enzymes, drug transporters and metabolising enzymes was measured using quantitative real-time PCR.

Results: We found that there was an increase in pregnane X receptor (PXR), a key regulator of drug transporters, in the PR liver in late gestation. However, there was decreased mRNA expression of multidrug resistance-associated protein 2 (MRP2), an efflux transporter located at the cannilicular membrane of hepatocytes, and organic anion transporting polypeptide C (OATPC), an uptake transporter located at the basolateral membrane of hepatocytes in both PR fetuses and lambs. PR also decreased mRNA expression of Cytochrome P450 1A2 (CYP1A2) in the liver of 21d lambs. However, LGON did not change the gene expression of drug transporters. Similar to the PR cohort, LGON decreased CYP1A2 mRNA expression in the liver of 30d lambs.

Conclusions: This study suggests that PR may alter fetal and neonatal drug disposition. However, LGON may have less of an effect on fetal and neonatal drug disposition.

Epigenetic regulation of CRH expression in human trophoblasts

X. Pan[2,3], J. Fitter[2,3], M. Bowman[2,3], R. J. Scott [2,3,4], R. Smith[2,3], T. Zakar[1,2,3]

[1]Obstetrics & Gynaecology, John Hunter Hospital, Newcastle, Australia; [2] Hunter Medical Research Institute, Newcastle, Australia; [3]Faculty of Health and Medicine, The University of Newcastle, Callaghan, Australia; [4]Molecular Medicine, HAPS, New Lambton Heights, Australia.
Tamas.Zakar@newcastle.edu.au

Background: Corticotropin Releasing Hormone (CRH) concentration increases in the maternal plasma during pregnancy, and the trajectory of the increase predicts gestational length. We have reported previously that the CRH promoter is methylated partially and allele-independently in trophoblasts, and that promoter epialleles with particular methylation patterns are activated selectively by cAMP in primary human trophoblasts.

Aims/Hypothesis: Our aim here was to determine whether histone modifications are involved in the epigenetic regulation of CRH gene activity in trophoblast cells.

Methods: Primary cultures of human trophoblasts were stimulated with 8-Br-cAMP. Histone modifications at the CRH proximal promoter were determined by chromatin immunoprecipitation. CRH promoter epialleles in DNA purified from the immunoprecipitated chromatin fractions were determined by clonal bisulfite sequencing.

Results: 8-Br-cAMP treatment robustly increased CRH expression. Histone-3 acetylation (H3ac), which activates genes, increased at the CRH promoter in trophoblasts during syncytialisation in culture, but there was no further increase in response to 8-Br-cAMP. H3ac was detected only at promoter epialleles responsive to 8-Br-cAMP or binding phospho-CREB. The level of other gene activating modifications such as histone-4 acetylation (H4ac) and histone-3, lysine-4 trimethylation (H3K4me3) also increased during syncytialisation and even further in response to 8-Br-cAMP, but these modifications targeted CRH promoter epialleles not involved in the 8-Br-cAMP-evoked transcriptional response. Histone-3-lysine 27-trimethylation and histone-3 lysine-9 trimethylation, which are repressive modifications, were reduced at the CRH promoter during syncytialisation and 8-Br-cAMP stimulation with little or no epiallele selectivity.

Conclusions: CRH gene expression in human trophoblasts is epiallele selective and is controlled by the pattern, rather than the extent, of promoter DNA methylation. CRH gene copies that carry promoter methylation patterns compatible with expression are opened up by histone-3 acetylation when trophoblasts syncytialise. These CRH epialleles respond to cAMP stimulation. CRH gene copies marked by H4ac and H3K4me3 do not carry H3ac marks and promoter methylation patterns required for expression. These epialleles are not responsive to cAMP stimulation. The number of inducible CRH gene copies is determined by the distribution of CRH promoter epialleles. Conditions that impact on DNA methylation may influence the number functional CRH gene copies and maternal CRH levels in normal and abnormal pregnancies.

Developing a method to visualise the endocrine pancreas using whole-slide imaging

Emma Buckels[1], Mark Oliver[1], Frank Bloomfield[1], Anne Jaquiery[1, 2]

[1]Liggins Institute, University of Auckland, Auckland, New Zealand; [2]Department of Paediatrics: Child and Youth Health, University of Auckland, Auckland, New Zealand.
e.buckels@auckland.ac.nz

Background: Historically, immunohistochemistry (IHC) stained tissue sections have been visually assessed for one or two proteins, with images collected at only a few fields of view (FOV) per slide to infer data for the entire tissue section. Technological advances are driving the adoption of whole-slide imaging (WSI) and antibody multiplexing in basic science research. WSI allows rapid scanning and creation of a digital image of the entire slide for multiple antigen targets. The ability of WSI to image entire tissue sections is capable of eliminating several sources of potential sampling bias, historically introduced by imaging smaller areas of tissue. These include within-section variability from randomly selected FOVs, introduction of observer bias in the selection of FOVs, or random sampling error.

Aims/Hypothesis: To describe use of the MetaSystems VSlide microscope scanner for imaging ovine pancreatic tissues stained with a multiplexed IHC protocol to assess endocrine cell numbers.

Methods: The pancreas was collected at post mortem from 48 adult sheep aged 18-months, stored in PFA and embedded in paraffin. 100 tissue sections at 5 µM thick were cut per animal. Five slides at 100 µM intervals underwent multiplexed staining for insulin, glucagon, somatostatin and DAPI. Slides were imaged using a Zeiss AxioImager Z2 microscope equipped with the MetaSystems VSlide slide scanner. Digital slides will be analysed using the MetaMorph Multi Wavelength Cell Scoring module, to characterise the physical characteristics of pancreatic α-, β- and δ-cells.

Results: Approximately 250 slides were imaged in total. Between 450-600 FOV were imaged per slide, with each slide taking 35-45 minutes to scan. Digital slides were 1.3-1.8 GB each.

Conclusions: Compared to traditional microscopy, WSI offers the ability to rapidly generate a digital image of each pancreas slide, increased the amount of data-per-slide generated, and reduced hands on time required. Issues arose over microscope use charges (approximately $9500NZD), digital slide storage and computational power required to analyse large files. The use of WSI has potential clear advantages over traditional manual slide imaging.

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Session 5

Chairs – Jane Pillow and Alistair Gunn			
11:30	**A25**	Gert S Maritz	Effect of maternal nicotine exposure on lung development: An overview
12:00	**A26**	Ali Hani (E)	Neonatal hyperoxia affects macrophage phenotype in mice: effectiveness of mesenchymal stem cell therapy
12:12	**A27**	Ruhan Kruger (U)	The effect of maternal whooping cough vaccination on fetal development and postnatal behaviour in spiny mice
12:24	**A28**	Stacey Ellery (ECR)	A pilot study of maternal creatine treatment in primate pregnancy- protecting the fetus from late gestation brain injury
12:36	**A29**	Yan Yee Chan (Hons)	Optimising the dose of erythropoietin required to prevent ventilation-induced brain injury
12:48	General discussion		

Effect of maternal nicotine exposure on lung development: An overview

Gert S Maritz and Jihaan Adonis

Department of Medical Biosciences, University of the Western Cape, Cape Town, South Africa.
gmaritz@uwc.ac.za

Background: Several studies showed that maternal smoking/nicotine exposure during gestation and lactation interferes with lung development in the offspring.

Aims/Hypothesis: The aim of the project was to investigate, 1)the effect of maternal nicotine exposure on lung development in the offspring from the early neonatal phase up to adulthood, 2) whether these effects can be prevented by restoring the mother's oxidant and ant-oxidant balance, and 3) whether the effect of maternal nicotine exposure on the offspring is transgenerational..

Methods: Pregnant rats (F0) received 1mg nicotine/kg body weight/day subcutaneously. The offspring (F1) were killed at postnatal days 14, 21, 42 and 84. Mating of the F1 females and males were allowed to generate the F2 generation. Lung tissue was removed for metabolic and histology. For structural studies the lungs were fixed at a transpulmonary pressure of 25 cm water for 30 minutes. Sections were made for histology, immune histology and morphology and stained for the appropriate tests. For metabolic studies tissue samples were kept on ice and as quickly as possible processed for investigating glucose turnover and enzyme activity.

Results: The data showed that maternal nicotine exposure had no effect on the body weight and litter size of the offspring. Glycolysis and glycogenolysis was suppressed due to inhibition of phosphofructokinase and phosphorilase respectively. Glucose flux through the hexose monophosphate shunt was markedly increased. No clear structural changes were observed at postnatal days 14 and 21. Structural differences appeared from postnatal day 42 and some only became evident at postnatal day 84. Emphysematous lesion became evident from postnatal day 42 and further deterioration was observed at postnatal day 84. These structural changes were followed by an increase in lung compliance. These changes were prevented by giving the mother tomato juice during pregnancy and lactation.

Analysis of the cell dynamics in the alveolar wall shows premature senescence and slower cell proliferation of particularly, the fibroblasts.

The emphysematous lesions were transferred from the F1 generation to the F2 generation.

Conclusions: Maternal nicotine exposure during gestation changes the program that control development of the lungs of the F1 offspring which is again transferred to the F2 generation. This is prevented by restoring the mother's oxidant-anti-oxidant balance.

Neonatal hyperoxia affects macrophage phenotype in mice: effectiveness of mesenchymal stem cell therapy

Ali Hani, Andrea Wise, Foula Sozo, Robert De Matteo, Richard Harding and Sharon D Ricardo

Department of Anatomy and Developmental Biology, Monash University, Melbourne, Australia
ali.hani@monash.edu

Background: Exposure to high levels of oxygen (hyperoxia) after birth can permanently injure the developing lungs, increasing the risk of poor lung function later in life. At present, there are no treatments for hyperoxia-induced lung injury. Pulmonary macrophages are a heterogeneous type of cell known to be involved in both lung injury and repair. Following exposure of the lung to injurious conditions, M1 macrophages promote inflammation, whereas M2 macrophages promote repair and regeneration. Macrophages can reversibly transfer between M1 and M2 phenotypes. Mesenchymal stem cells (MSCs) are thought to promote macrophages changing from a M1 to M2 phenotype, which could be beneficial in promoting lung repair following exposure to neonatal hyperoxia.

Aims/Hypothesis: Our aims are to investigate how (a) supplemental oxygen in the period after birth affects myeloid cells, including macrophages, in the developing lung immediately after exposure, and (b) MSC administration following hyperoxia affects myeloid cell populations, including changes in macrophage phenotype.

Methods: Newborn mice were exposed to 90% O_2 (hyperoxia) or 21% O_2 (normoxia) from postnatal (P) day 0 to P4. In a subset of hyperoxia-exposed mice, a single intranasal injection of $2.5X10^5$ human MSCs in 70µl PBS was given to each pup at P4, after which they breathed room air until P7. Mice were killed at P0, P4 and P7 (n=8/group). Lungs were collected, digested with collagenase and DNase in RPMI for 45 min at 37ºC and the red blood cells were then lysed. The total cell number was counted and $3X10^6$ cells were allocated from each lung for staining with an antibody cocktail (CD45, CD11b, CD11c, Ly6C, Ly6G, F4/80 and CD206). Myeloid cell populations ($CD45^+$) within the digested lungs were examined using flow cytometry.

Results: At P4, total lung cell counts were significantly decreased ($P<0.05$) by hyperoxia. Hyperoxia resulted in a significant increase in $Ly6C^{+low}/Ly6G^+$ granulocytes; however, $CD11b^{+hi}/CD11c^+$ and $F4/80^+$/intracellular $CD206^+$ (M2) macrophages were significantly decreased ($P<0.05$). At P7, the hyperoxic lungs had an increase in $CD11b^{+hi}/CD11c^+$ macrophages; MSC administration following hyperoxia significantly increased $F4/80^+/CD206^+$ M2 macrophages.

Conclusions: At P4 and P7, neonatal hyperoxia resulted in altered myeloid cell populations within the lung, however intranasal administration of human MSCs following hyperoxia appeared to alter macrophage phenotype, which could promote tissue repair.

The effect of maternal whooping cough vaccination on fetal development and postnatal behaviour in spiny mice

Ruhan Kruger, Hayley Dickinson, Nadia Bellofiore, David Walker, Tim Moss

The Ritchie Centre, Hudson Institute of Medical Research; Department of Obstetrics and Gynaecology, Monash University, Clayton, Victoria, Australia.
rfkru1@student.monash.edu

Background: Whooping cough is a potentially fatal infectious respiratory disease caused by *Bordetella pertussis* bacteria. Despite a well-established vaccination program, the incidence of whooping cough in Australia remains unacceptably high. Children under 6 months of age are most at risk of infection because of the time taken to achieve immunity after initiating postnatal vaccination. Thus, vaccination of pregnant women early in the third trimester of pregnancy has recently been implemented to protect infants against whooping cough from the time of birth. Limited clinical data show no adverse effects of maternal pertussis vaccination in pregnancy. There have been no detailed examinations of the effects of maternal pertussis vaccination on fetal development or postnatal outcomes in humans or experimental animals, despite the established capacity of maternal immune activation by other means to alter fetal brain development and postnatal behaviour.

Aim: To determine the effect of maternal vaccination against whooping cough on neurodevelopment in fetal and postnatal spiny mice.

Methods: Pregnant spiny mice receive a subcutaneous injection of combined Diphtheria-Tetanus-acellular Pertussis vaccine (Boostrix®, GSK; 0.125ml: n=15) or saline (0.125ml: n=15) on day 24 of gestation (term is 39 days). Maternal blood, placenta and fetal tissues are collected on day 37 of gestation (n=6/group) to determine effects on fetal growth and neurodevelopment. The remaining 9 dams in each group are left to give birth spontaneously: after weaning the offspring undergo a battery of neurobehavioural tests prior to tissue collection.

To date, 12 pregnant spiny mice have received experimental treatment (n=6/group). Tissues have been collected from 4 pregnancies (2 Boostrix, 2 control) at 37 days of gestation. Analysis of covariance (with dam as a covariate) was used to compare fetal body and organ weights between Boostrix (n=8 fetuses) and saline (n=7) groups.

Results: There have been no complications from experimental treatments. Fetal body weights were different between Boostrix and control groups (p=0.036). Data from a further 4 pregnancies (2 Boostrix, 2 control) will be available by the time of abstract presentation.

Conclusions: Our initial data may not accurately reflect the effects of maternal Boostrix administration. Neurodevelopmental assessments in children born after maternal whooping cough vaccination will not be obtained from human studies for some time. Thus, this project may provide reassurance of the safety of maternal pertussis vaccination, or timely identification of the potential for neurodevelopmental problems in children of immunised mothers.

A pilot study of maternal creatine treatment in primate pregnancy-protecting the fetus from late gestation brain injury

Stacey Ellery[1,2], Meredith Kelleher[3], David Walker[1,2], Hayley Dickinson[1,2], Rod Snow[4] & Peta Grigsby[3]

[1]The Ritchie Centre, Hudson Institute of Medical Research, Melbourne, Australia; [2] Department of Obstetrics and Gynaecology, Monash University, Melbourne, Australia; [3]Oregon National Primate Research Center, Oregon Health and Sciences University, Portland, USA; [4]Institute for Physical Activity and Nutrition, Deakin University, Melbourne, Australia.
stacey.ellery@hudson.org.au

Background: We have extensive evidence to show that maternal dietary creatine supplementation significantly reduces mortality and multi-organ morbidity in a rodent model of birth asphyxia. Through a collaboration with the Oregon National Primate Research Centre we are now conducting a pilot study of creatine in non-human primate (NHP) pregnancy.

Aims/Hypothesis: We aimed to gather preliminary data on: [1] the safety and efficacy of prolonged maternal dietary creatine supplementation for the mother and fetus during NHP gestation; and [2] if exposure to creatine *in utero* protects the fetal brain from the effects of an umbilical cord occlusion.

Methods: At 104-106 days gestation (d GA) (term ~165 days) rhesus macaques (n=4) were surgically instrumented with fetal ECG electrodes, and catheters inserted into the amniotic sac, maternal femoral vein and artery. This allowed for continuous monitoring of maternal blood pressure (BP) and fetal heart rate (HR), uterine contractility and routine sampling of maternal blood and amniotic fluid. From 115d GA NHPs received creatine orally (0.3g/kg/day for 8 days, followed by 0.075g/kg/day until delivery; n=2), or vehicle (apple sauce; n=2) as controls. Maternal blood, urine and amniotic fluid was sampled throughout supplementation, to assess creatine content, basal metabolic status, and renal and hepatic functions. At 147-148d GA one creatine-treated and one control fetus underwent a 12-minute umbilical cord occlusion (UCO), by cord clamping, to induce a hypoxic insult before caesarian delivery.

Results: Dietary creatine supplementation increased maternal plasma and amniotic fluid creatine levels by ~60%. Preliminary analysis suggests that creatine had no affect on maternal health parameters, including BP and uterine activity. After UCO the creatine supplemented neonate had a 30 min Apgar score of 8, compared to 2 for the control-UCO. The control-UCO infant displayed wrist flexion, reduced forelimb motor coordination and a reduced suckling reflex from birth until 5-days post term equivalent age, behaviours not observed in the creatine supplemented infant after UCO.

Conclusions: This pilot study suggests that maternal dietary creatine is safe for the NHP mother and fetus, and can reduce neonatal morbidity following an umbilical cord occlusion in late gestation. More extensive studies to address this hypothesis in the NHP are now in planning.

Optimising the dose of erythropoietin required to prevent ventilation-induced brain injury

Yan Yee Chan, Domenic LaRosa, Mary Tolcos, Anqi Li, Valerie Zahra, Samantha Barton and Graeme Polglase

The Ritchie Centre, Department of Obstetrics and Gynaecology/Pediatrics, Monash University and Hudson Institute of Medical Research, Clayton, Victoria, Australia.
yycha32@student.monash.edu

Background: Inadvertently injurious ventilation of preterm neonates in the delivery room can cause brain injury through cerebral inflammation and haemodynamic instability. Recombinant human erythropoietin (rhEpo) has been shown to be neuroprotective in preterm infants but its interaction with mechanical ventilation is not well understood.

Aims: We aimed to investigate the optimum doses of rhEpo to reduce ventilation-induced brain injury in preterm lambs.

Methods: Preterm lambs (0.85 gestation) were ventilated with an injurious strategy for 15 min followed by conventional ventilation for 105 min. Lambs were randomised to controls (VENT; n=8), or received a bolus injection of rhEpo (EPREX®) 300 IU/kg (EPO 300; n=5), 1000 IU/kg (EPO 1000; n=5), or 3000 IU/kg (EPO 3000; n=5) at 6±2 min. qRT-PCR and immunohistochemistry were used to assess brain inflammation, cell death, and vascular leakage in the periventricular and subcortical WM (PVWM; SCWM). One-way ANOVA was used for statistical analysis. Values of $p<0.05$ were considered statistically significant.

Results: All EPO lambs had detectable levels of rhEpo in cerebrospinal fluid 2 h following administration. Molecular and histological inflammatory indices in the PVWM were not different between groups. EPO 300 lambs had higher IL-6 mRNA expression in the SCWM than VENT lambs ($p=0.0055$). Cell death marker p53 mRNA levels were higher in all EPO groups compared to VENT ($p<0.01$ for all) in the PVWM but not different in the SCWM. Occludin mRNA levels were higher in EPO 3000 lambs than all groups in the PVWM, and compared to VENT lambs in the SCWM ($p=0.0448$). Claudin-1 mRNA levels in the PVWM were higher in EPO 3000 lambs compared to VENT ($p=0.0113$). Claudin-5 mRNA levels were lower in EPO 3000 lambs than VENT in the SCWM ($p=0.0194$). The number of blood vessels with protein extravasation in the SCWM was lower in EPO 1000 (vs VENT $p=0.01$) and EPO 3000 (vs VENT $p=0.0246$) lambs but not different between groups in the PVWM.

Conclusions: Early administration of rhEpo at doses of 300 IU/kg, 1000 IU/kg, and 3000 IU/kg did not exacerbate brain inflammation and injury resultant from mechanical ventilation. However, higher doses of 1000 and 3000 IU/kg rhEpo may provide neuroprotection by maintaining blood-brain barrier integrity.

Session 6

Chairs – Graeme Polglase and Kathy Gatford			
2:00	**A30**	Tim Moss	Preterm lung maturation: a cautionary tale and a new direction
2:20	**A31**	Tanzila Mahzabin (L)	The influence of antenatal steroid on preterm sheep diaphragm
2:32	**A32**	Amy Wooldridge (L)	Effects of maternal asthma on the fetal immune system
2:44	***A33**	Paris Papagianis (E)	The effect of postnatal steroids on the lung structure of ventilated preterm lambs exposed to chorioamnionitis
2:56	**A34**	Julia Shaw (L)	Maternal administration of progesterone: effects on neurosteroidogenesis and neurodevelopment
3:08	***A35**	Madison Paton (E)	Brain inflammation in preterm fetal sheep to examine the benefits of umbilical cord blood and cord tissue stem cell therapies
3:15	General discussion		

Preterm lung maturation: a cautionary tale and a new direction

Tim Moss

The Ritchie Centre, Hudson Institute of Medical Research; Department of Obstetrics & Gynaecology, Monash University, Clayton, Victoria, Australia.
tim.moss@monash.edu

Background:
Respiratory disease is the principal cause of illness and death in preterm infants. There are numerous pre- and postnatal factors that influence risk of preterm lung disease, which interact with other systems to influence long-term health. For example: intrauterine inflammation can precipitate preterm birth and adversely affect brain development, yet it reduces risk of neonatal respiratory distress syndrome (RDS); antenatal corticosteroid therapy induces lung maturation prior to preterm birth and reduces risk of RDS, but attempts at its optimisation have resulted in practices that may have life-long adverse consequences.

Aims:
My presentation will highlight work I performed in collaboration with Prof Richard Harding, aimed at understanding effects of antenatal corticosteroids and intrauterine inflammation on fetal development and postnatal physiological function.

Methods and Results:
We injected single or multiple doses of betamethasone to pregnant ewes or directly to fetal sheep, to examine effects on fetal growth and identify potential 'programming' effects. Maternal betamethasone injections reduced birth weight but direct fetal injections did not, and maternal (but not fetal) injections caused insulin resistance in adult offspring; however, maternal and fetal betamethasone injections – despite having differing effects on birth weight – reduced brain weight in preterm, term or adult sheep. Our findings from sheep experiments are consistent with observations in humans.

We investigated the effects on fetal lung and brain development of intrauterine inflammation by injecting lipopolysaccharide (LPS) into the amniotic sac in sheep. We observed inflammation and axonal disruption in the brains of fetal sheep exposed to intra-amniotic LPS. In independent experiments Richard's group showed more severe brain pathology from fetal intravenous LPS injections. My own independent experiments showed profound effects of intra-amniotic LPS on fetal lung development, including large increases in surfactant production by the preterm lungs.

Recent experiments by members of my group are using primary-cell culture techniques to identify mechanisms for inflammation-induced precocious surfactant production by the preterm lungs. We have shown that inflammation acts indirectly on type II pulmonary epithelial cells from fetal mouse lungs to induce surfactant production: pulmonary fibroblasts respond to an inflammatory stimulus by secreting a factor (or factors) that stimulates epithelial cell surfactant production. Attempts to identify the responsible factor(s) are ongoing.

Conclusions:
Our experiments in sheep show the potential for antenatal corticosteroid administration (particularly repeated doses) to have adverse effects, which might be consequences of the treatment regimen proposed in new clinical practice guidelines for the use of antenatal corticosteroids in women at risk of preterm birth. We hope our work understanding the mechanism(s) responsible for inflammation-induced precocious surfactant production will result in a new approach to prevent preterm lung disease that avoids adverse side-effects.

The influence of antenatal steroid on preterm sheep diaphragm

Tanzila Mahzabin, Yong Song, Kanakeswary Karisnan, Peter Noble, Gavin J.Pinniger, J. Jane Pillow

School of Anatomy, Physiology and Human Biology, The University of Western Australia (M309), 35 Stirling Highway, Crawley WA6009, Australia.
tanzila.mahzabin@research.uwa,edu.

Background: Pregnant women at high risk of preterm delivery receive glucocorticoids to accelerate fetal and lung maturation and surfactant synthesis. Antenatal steroids also reduce the risk of neonatal pulmonary morbidity and neonatal death. However the effect of antenatal steroids on the developing organ system is still unclear. Maternal steroids have no effect on the rat pup diaphragm until 21 day postnatal age, when decreased contractile force is evident.

Aims/Hypothesis: We aimed to determine how maternal betamethasone and duration of treatment alters the ovine fetal diaphragm at the functional and molecular level. We hypothesised that antenatal betamethasone would impair the function of the immature diaphragm by redirecting the protein homeokinetic process towards net protein catabolism inducing muscular atrophy and increasing susceptibility to fatigue and damage. We also speculated that fetal diaphragm atrophy would be more evident in lambs with longer duration of exposure to maternal betamethasone.

Methods: Date-mated merino ewes received intramuscular saline (control) or betamethasone (0.15 mg/kg at 24 hour interval) at 2 d and 14 d prior to delivery at 121 d gestational age (term= 150 d). Preterm fetal lambs were euthanized at delivery. The right hemi-diaphragm was used for *in vitro* contractile measurements. The left costal diaphragm was snap frozen for molecular analysis. The expression of myosin heavy chain (MHC) isoforms and atrophy related genes, protein metabolic pathway and oxidative stress were assessed using quantitative PCR, western blot and biochemical assay.

Results: The contractile properties of the fetal ovine diaphragm were unchanged by antenatal betamethasone exposure. The protein synthesis pathway was down regulated via depressed expression of mTOR and Ps6K proteins. A 2 d betamethasone exposure increased 20S proteasome activity significantly however 20S proteasome activity was down regulated at 14 d exposure compared to control. Protein oxidation was up regulated in betamethasone treated samples accompanied by increased expression level of antioxidant genes catalase and sod1 compared to control.

Conclusions: Antenatal betamethasone likely contributes to fetal diaphragm muscle atrophy by inhibiting the anabolic pathway rather than by activating proteolysis. Fetal diaphragm atrophy after betamethasone results primarily from depressed protein synthesis, with an additional contribution from oxidative stress. However different durations of betamethasone exposure do not seem to have any differential effect on fetal diaphragm at the functional and molecular levels.

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Effects of maternal asthma on the fetal immune system

Amy Wooldridge[1], Kathryn Gatford[1], Tim Moss[2,3], Courtney McDonald[2], Vicki Clifton[1,5], Rob Bischof[2,4]

[1]Robinson Research Institute and School of Medicine, University of Adelaide, Adelaide, Australia; [2]Hudson Institute of Medical Research, [3]Department of Obstetrics and Gynaecology, [4]Department of Physiology, Monash University, Clayton, Australia; [5]Mater Medical Research Institute, University of Queensland, Brisbane, Australia.
amy.wooldridge@alumni.adelaide.edu.au

Background: Maternal asthma affects up to 12% of human pregnancies and is associated with increased risk of allergy in offspring. The mechanisms for programming of allergy risk in progeny by maternal asthma are unknown, and were therefore investigated in our novel model of maternal allergic asthma in ovine pregnancy[1].

Aims/hypothesis: To examine how induced maternal allergic asthma in sheep affects the fetal immune system.

Methods: Allergic asthma was induced in sheep before mating by sensitisation to house dust mite (HDM), and serial airway challenges with HDM continued throughout pregnancy. Controls received saline. IgE levels in maternal and fetal serum and amniotic fluid were measured by ELISA. Thymic and splenic lymphocyte phenotypes (CD4, CD8, CD5, CD44) from singleton fetuses gestated in allergic (n=7) or control (n=5) sheep were examined by flow cytometry.

Results: Relative fetal weights were decreased by 12% within the maternal allergic asthma experimental group (P=0.038)[1]. HDM-specific IgE levels were elevated in serum of allergic compared to control ewes but undetected in fetal serum or amniotic fluid samples. Weights of fetal immune tissues were similar between experimental groups. There were no changes in the proportions of CD4+, CD8+, CD4-CD8-, CD4+CD8+ or CD5+ thymocytes or splenocytes collected from fetuses gestated in allergic compared to those from control sheep. However, the proportion of $CD44^+$ thymocytes was increased in fetuses of allergic compared to control ewes (Control 4.08 ± 3.09%, Allergic 14.23 ± 11.69%, P=0.032), though CD44+ splenocytes did not differ.

Conclusions: In late gestation we detected an increase in CD44 expression, suggestive of increased thymocyte activation in fetuses from allergic compared to control ewes. We are currently exploring the effects of this exposure on immune cells within the lung.

1. Clifton VL, Moss TJ, Wooldridge AL, Gatford KL, Liravi B, Kim D, Muhlhausler BS, Morrison JL, Davies A, Matteo R, Development of an experimental model of maternal allergic asthma during pregnancy. Journal of Physiology 2016. 594(5), 1311-25.

The effect of postnatal steroids on the lung structure of ventilated preterm lambs exposed to chorioamnionitis

Papagianis PC[1,2], Noble PB[2], Ahmadi-Noorbakhsh S[2], Moss TJ[1], Pillow JJ[2]

[1]The Ritchie Centre, Hudson Institute of Medical Research, Melbourne; [2]Anatomy, Physiology & Human Biology & Centre for Neonatal Research & Education, University of Western Australia, Perth.
paris.papagianis@monash.edu

Background: Lung inflammation is central to the pathogenesis of bronchopulmonary dysplasia (BPD), a chronic lung disease of preterm infants. Lung inflammation impairs alveolarisation, which is a characteristic pathological feature of BPD. Glucocorticoids are anti-inflammatory, improve lung function, and reduce ventilator requirements in preterm infants. However, glucocorticoids inhibit formation of secondary septa in naïve lungs, thus inhibiting alveolarisation. The effect of glucocorticoids on alveolarisation in preterm newborns exposed to antenatal inflammation is unknown.

Aims: To determine the effects on lung structure of early postnatal dexamethasone administration in preterm lambs exposed to inflammation *in utero*.

Methods: Fourteen pregnant ewes received intra-amniotic lipopolysaccharide (LPS from E.coli 055:B5; 4mg/mL) at 126 days of gestation (term ~150 d). Preterm lambs were exteriorised, intubated and delivered by Caesarean section, prior to resuscitation and initiation of mechanical ventilation. Lambs were weaned using a graded de-escalation of respiratory support. Lambs received either a tapered course of intravenous dexamethasone (n=7: 0.15 mg/kg/d for 3 d, 0.1 mg/kg/d for 2 d and 0.05 mg/kg/d for 2 d) or equivalent volumes of saline (n=7), commencing within 3 h after birth. Lambs were killed for tissue collection at 7 d. The left lung was fixed and processed: one section (5 μm) from the left lower lobe of each lamb was stained with Hart's elastin stain. Secondary septae were identified by elastin deposition at the tips of tissue extending into the airspace. Mean tissue and septal crest fractions were determined from 3 randomly chosen fields of view using a point-counting grid. All measurements were made by a single assessor, blinded to the treatment group. Blinding was preserved in statistical analyses (t-test) to preserve integrity of future assessments.

Results: The septal crest fraction was significantly different between groups (3.3 ± 0.9 % vs. 0.6 ± 0.2 %; p=0.007). The tissue fraction was not different between groups (52.6 ± 4.4 % vs. 50.5 ± 2.6 %; p=0.86).

Conclusions: Early postnatal dexamethasone alters septation in the lungs of preterm lambs exposed antenatally to inflammation. Further histological measures will identify the effect of dexamethasone on other indices of lung development and injury resulting from postnatal ventilation and antenatal inflammation. Biomolecular measurements will identify the effects of early postnatal dexamethasone on lung inflammation and mediators of lung development and injury in these lambs.

Maternal administration of progesterone: effects on neurosteroidogenesis and neurodevelopment

Julia Shaw[1,2], Hannah Palliser[1,2], Poonam Rani[1,2], and Jonathan Hirst[1,2]

[1] School of Biomedical Sciences and Pharmacy, University of Newcastle, Newcastle, Australia
[2] Mothers and Babies Research Centre, Hunter Medical Research Institute, Newcastle, Australia.
Julia.shaw@uon.edu.au

Background: Use of progesterone to prevent preterm labour is increasing, however the effects of changing steroid hormone profiles on the developing fetus are not clear. Progesterone treatment may influence the production and levels of neuroactive metabolites, which may then alter normal brain development, as these steroids are known to have major roles in neurodevelopment.

Aims/Hypothesis: We hypothesize that progesterone administration will change normal neurosteroidogenensis in the placenta and fetal brain, and potentially have adverse effects on brain development which may occur in a sex-dependant manner. Therefore, our aim is to evaluate the effects of progesterone treatment used in pregnancy on the production and levels of key neuroactive steroids in the fetus and to assess the effects on preterm brain development.

Methods: Pregnant guinea pig dams were administered progesterone (5mg/kg) or vehicle (45% β-cyclodextrin) orally daily from GA29-60. Fetal tissues and plasma were collected at GA61 (preterm). Maternal saliva was collected weekly throughout pregnancy and progesterone levels were measured by enzyme immunoassay. Maternal and fetal plasma allopregnanolone concentrations were determined by radioimmunoassay, and the Hunter Area Pathology Service measured fetal plasma cortisol concentrations. Glial fibriallary acidic protein (GFAP) staining was used to examine reactive astrocyte expression in the CA1 region of the hippocampus and the dentate gyrus. Placental expression of key enzymes including the 5α-Reductases was also be analysed by western blotting.

Results: As expected, maternal salivary progesterone was higher for those receiving progesterone compared to vehicle (GA35-60) ($p<0.0001$). Maternal plasma allopregnanolone concentration trended towards an increase following progesterone treatment, as did the plasma concentration for the pups from progesterone-treated dams. Males from progesterone-treated dams had lower GFAP expression in the CA1 region of the hippocampus ($p=0.04$), whereas females from progesterone-treated dams had lower expression in the dentate gyrus ($p=0.0009$). Unexpectedly, this was not due to an increase in cortisol, as fetal plasma cortisol concentrations were unaffected by progesterone treatment. Placental data protein data was also analysed.

Conclusions: Administration of progesterone during pregnancy appears to have differing adverse effects on the brain development of preterm males and females. This does not appear to be due to an increase in cortisol, but potentially due to elevated levels of allopregnanolone or other progesterone metabolites, which are yet to be fully investigated.

Brain inflammation in preterm fetal sheep to examine the benefits of umbilical cord blood and cord tissue stem cell therapies

Madison Paton[1], Courtney McDonald[1], Beth Allison[1], Michael Fahey[1,3], Amy Sutherland[1], Jamie Mihelakis[1], Ilias Nitsos[1], Dalibor Stanojkovic[1], Tamara Yawno[1], Graham Jenkin[1,2] and Suzie Miller[1,2]

[1]The Ritchie Centre, Hudson Institute of Medical Research, Clayton, Australia, [2]Department of Obstetrics and Gynaecology, Monash University, Clayton, Australia, [3]Monash Children's, Monash Health, Department of Paediatrics, Clayton, Australia.
madison.paton@hudson.org.au

Background: Improvements in medical care for preterm newborns have increased survival rates, however prematurity still accounts for up to 70% of perinatal deaths and 50% of poor neurologic outcome and cerebral palsy. Of these preterm babies, 40-70% have been exposed to an inflammatory condition affecting the placenta and its membranes, termed chorioamnionitis. Chorioamnionitis leads to fetal inflammation and infection, and has damaging effects on the brain. My PhD studies examine white matter injury in a preterm model of lipopolysaccharide (LPS)-induced inflammation, and explore the beneficial effects of stem cells isolated from umbilical cord tissue and umbilical cord blood.

Aims/Hypothesis: To induce brain inflammation and white matter injury in preterm fetal sheep, and to examine the potential benefits of specific cord tissue and cord blood stem cell populations. We hypothesised that LPS administration at 0.65 gestation to fetal sheep causes clinically relevant neuroinflammation and white matter injury, and that cellular therapies will attenuate this injury.

Methods: Ewes carrying a singleton preterm 0.65 gestation fetus were surgically instrumented with catheters followed by a 4-day recovery period. Animals received LPS (055:B5, 200ng) or saline IV daily for 3 days. Fetal plasma was collected before LPS or saline administration and 1, 3, 6 and 12 hours after every LPS/saline dose. On day 10, animals were euthanised, with brains collected for PCR and histology.

Results: To date we have collected n=3 LPS exposed preterm fetal sheep and observed gross white matter damage with brains being more gelatinous and fragile, compared to control fetuses (n=3). LPS treated fetal sheep had higher levels of infiltrating cells within the white matter and cortex compared to controls.

Conclusions: Preterm brain inflammation in response to fetal LPS exposure in pregnant sheep, resulted in significant fetal cerebral changes in the white matter and cortex. Future studies will evaluate the role of specific umbilical cord blood and cord tissue derived stem cells in reducing this damage following preterm brain inflammation.

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Session 7

Chairs – Barbara Lingwood and Karen Moritz			
4:00	**A36**	Mary Wlodek	Cardiorenal and metabolic risk for offspring born small: impact of lifestyle and its consequences on the next generation
4:10	**A37**	Jane Black	Consequences of preterm birth on the immature renal and cardiovascular systems
4:30	**A38**	Ian Wright	Hydrogen sulphide production capacity in the perinatal heart
4:42	**A39**	Sarah Walton (L)	Prenatal hypoxia combined with a high-salt diet increases risk of renal and cardiovascular impairments in adult mice
4:54	**A40**	Yvonne Eiby	Pilot trial of early blood transfusions for supporting cardiovascular function and cerebral oxygen delivery in preterm piglets
5:06	**A41**	John Bertram	Maternal low protein diet leads to low podocyte endowment
5:18	General discussion		

Consequences of preterm birth on the immature renal and cardiovascular systems

M.J. Black

Department of Anatomy and Developmental Biology, Monash University, Clayton, Victoria, Australia.
Jane.black@monash.edu

Background: Preterm birth (defined as birth prior to 37 weeks of gestation) occurs in approximately 10% of all births worldwide. Preterm infants are born at a time when their organs are structurally and functionally immature. In relation to the renal and cardiovascular systems, preterm infants are born at a time when nephrogenesis is ongoing in the kidney, cardiomyocytes of the heart are relatively immature and the walls of blood vessels are underdeveloped. Over the past decade we have been conducting human and animal studies (using a clinically relevant ovine model of preterm birth) to address the question: what is the effect of preterm birth and its antecedents on the growth and structure of the kidneys, heart and vasculature? Of concern, we have shown that preterm birth is often associated with marked glomerular abnormalities in the outer renal cortex. Intrauterine growth restriction and chorioamnionitis, both common antecedents of preterm birth can also adversely impact nephrogenesis. As expected, we have confirmed that the heart muscle is relatively immature at birth; however, following premature birth there is often rapid cessation of cardiomyocyte proliferation as well as maladaptive remodelling of the myocardium. Exposure to chorioamnionitis in utero also alters the growth of the cardiomyocytes. In the conduit arteries we have shown marked remodelling of the aortic and pulmonary artery wall following preterm birth and of concern, there is arterial injury in the ascending aorta.

Overall, the structural changes we have observed in the kidneys, heart and blood vessels following preterm birth are likely to predispose to long-term cardiovascular disease.

Hydrogen sulphide production capacity in the perinatal heart

Rebecca M Dyson[1,2], Nicole Wilding[1], Hannah Palliser[3], Megan Kelly[1], Ian M R Wright[1,3]

[1]Illawarra Health and Medical Research Institute, University of Wollongong, Gwynneville, Australia; [2]BRU, University of Otago, Wellington, New Zealand; [3]Hunter Medical Research Institute, University of Newcastle, New Lambton Heights, Australia.
iwright@uow.edu.au

Background: Significant cardiovascular dysfunction in the first days of life predicts mortality and morbidity in the preterm, with both peripheral microvascular tone and cardiac function implicated. Total body turnover of the gasotransmitter hydrogen sulphide (H_2S) is increased in those at greatest risk and H_2S is increased in failing adult hearts. H_2S production capacity of perinatal heart tissue and the relative contributions of the 3 main sources; Cystathionine-β-synthase, Cystathionine-γ-lyase, 3-mercaptopyruvate sulfurtransferase (CBS, CSE, MST), are unknown.

Aims/Hypothesis: It was hypothesised that the perinatal heart would have significant capacity to produce hydrogen sulphide. This study aimed to show this capacity and the contribution of the individual metabolic pathways in the term guinea pig pup, as well as the effect of age (fetal to 24 hours) and any sex differences.

Methods: Western Blot analysis was undertaken for CBS and MST protein expression and normalised to β-actin for perinatal (fetal, 10 hr and 24 hr) heart samples from term guinea pig pups of both sexes. Enzyme activity was measured in fetal and 24 hr heart samples from the same pups. Maximum H_2S production rate from crushed tissue, with excess substrate and co-factors, was recorded using a micro-respiration ion-specific electrode system (Unisense) and then repeated in the presence of specific inhibitors (PAG and AOAA) to delineate the different enzyme contributions.

Results: Both CBS and MST are expressed in the term perinatal guinea pig heart but neither expression significantly changed after birth nor between the sexes. On enzyme activity assay CSE and CBS were the predominant enzymes, with MST activity only detected in fetal male samples. CBS activity was lower in fetal female samples which led to a significant increase in females from fetal to 24 hrs ($p=0.02$). The levels of CSE were higher in male fetal animals, in the presence of sustained CBS activity and some MST, leading to a higher total capacity for H_2S production (1627microM/h/g) than any other subgroup ($p=0.001$). Overall CBS contributed between 54 and 60% of cardiac H_2S production capacity.

Conclusions: This study shows, for the first time, that the term guinea pig heart has the capacity to produce the gasotransmitter H_2S and the enzyme pathways that predominate (CBS>CSE>>MST). Some sexually dimorphic changes were observed. The exact location, physiological role and regulatory triggers remain to be delineated. These studies will allow further studies of the preterm animal and thus potential future interventional strategies.

Prenatal hypoxia combined with a high-salt diet increases risk of renal and cardiovascular impairments in adult mice

Sarah L Walton[1], Reetu R Singh[2], Joan Li[3], Helle Bielefeldt-Ohmann[4], Tamara M Paravicini[1], Melissa H Little[3] and Karen M Moritz[1]

[1]School of Biomedical Sciences, The University of Queensland, Brisbane, Australia; [2]Department of Physiology, Monash University, Melbourne, Victoria; [3]Institute for Molecular Bioscience, The University of Queensland, Brisbane, Australia; [4]School of Veterinary Science, The University of Queensland, Brisbane, Australia.
sarah.walton@uqconnect.edu.au

Background: Impaired oxygen supply to the fetus in a common clinical complication during pregnancy. Previously we have shown that prenatal hypoxia reduces nephron number and increases blood pressure in male, but not female mouse offspring.

Aims/Hypothesis: This study aimed to evaluate the combined impact of prenatal hypoxia and a postnatal high-salt diet on renal and cardiovascular development and function in the mouse. We hypothesised that prenatal hypoxia would impair kidney development, predisposing offspring to renal and cardiovascular disease. Furthermore, these impairments would be exacerbated by a high-salt diet.

Methods: Pregnant CD1 mice were housed in a hypoxic chamber (12.0% O_2) or control (21% O_2) environment from embryonic day 14.5 to 19.5 (birth). Offspring consumed control (0.2% NaCl) or high-salt diets (5% NaCl) from 10 weeks to 12 months of age. Renal function was examined via 24h metabolic cages. Mesenteric arteries were collected for pressurised in vitro myography studies and thoracic aorta sections were stained with Masson's Trichrome and Verhoeff's van Gieson stain for elastin. Kidney sections were evaluated by an expert pathologist.

Results: Chronic high salt intake increased kidney and heart mass in all animals, irrespective of prenatal treatment. Kidney sections from male hypoxia-exposed offspring showed glomerular hypertrophy and glomerulosclerosis compared to male control offspring. Histopathological changes were markedly exacerbated by the high-salt diet. In contrast, female hypoxia-exposed offspring displayed no overt signs of renal impairment or histopathology. Male and female hypoxia-exposed offspring both presented with mild vascular endothelial dysfunction. Consumption of a high-salt diet in both sexes led to marked mesenteric vascular stiffening and significant alterations to collagen and elastin deposition in the thoracic aorta in hypoxia-exposed offspring.

Conclusions: In summary, prenatal hypoxia perturbed kidney development and increased susceptibility to salt-induced renal injury in male offspring. Both sexes developed signs of cardiovascular disease in adulthood, suggesting the renal system of female mice was protected from an *in utero* hypoxic insult.

Pilot trial of early blood transfusions for supporting cardiovascular function and cerebral oxygen delivery in preterm piglets

Yvonne Eiby[1,2], Tracey Bjorkman[1,2], Julie Wixey[1,2], Kirat Chand[1,2], Paul Colditz[1,2], Barbara Lingwood[1,2]

[1]Perinatal Research Centre, School of Medicine, The University of Queensland, Brisbane, Australia; [2]UQ Centre for Clinical Research, The University of Queensland, Brisbane, Australia.
y.eiby@uq.edu.au

Background: Preterm infants are at high risk of brain injury. Poor cardiovascular function on the first day of life is a major contributor to preterm brain injury but current treatments aiming to reduce brain injury by supporting cardiovascular function do not improve preterm outcomes. The first-line treatment, volume expansion using saline, is ineffective in 40% of babies and is not associated with improved outcomes.

Aims/Hypothesis: We hypothesised that early blood transfusion would be more effective than saline for supporting cardiovascular support, increasing brain oxygenation, and reducing brain injury.

Methods: Preterm piglets (*n*=5/group) were delivered by caesarean section at 97/115d (developmentally similar to very preterm human infants born at 27wk gestation). Piglets were maintained under standard NICU conditions and randomised to either blood transfusion or saline infusion if mean arterial blood pressure (BP) was <27mmHg (common NICU definition of hypotension and threshold for clinical volume expansion) at the end of 30min baseline. Blood or saline (10mL/kg given i.v. over 30min) treatment was repeated up to 3 times if hypotension persisted/reoccurred. Measurements included BP, cerebral total oxygenation index (TOI) using near infra-red (NIRS) monitor, ventilation parameters, and arterial lactate levels. Piglets were euthanized 4h after commencement of treatment (~10h after birth). Brain injury was assessed using early markers of brain injury, including gene expression of inflammation markers IL-1β, IL-4, IL-6, TNFα, and TGFβ2.

Results: Piglets who received blood transfusions had significantly increased BP and TOI compared to baseline, and had a reduction in ventilation requirements (inspired O_2 and peak inspiratory pressures). Saline treatment did not significantly increase BP or TOI, and ventilation requirements at the end of treatment were significantly greater than piglets in the blood transfusion group. Expression of pro-inflammatory cytokines, IL-1β, IL-6 and TNFα, was significantly upregulated in saline infused piglets compared to brain tissue collected from littermates at birth. The same cytokines showed either a much smaller increase or a small decrease following blood transfusion. The anti-inflammatory cytokine IL-4 was upregulated following blood transfusion but not saline treatment, while TGFβ2 was downregulated following saline treatment.

Conclusions: Early blood transfusions are more effective than the current form of volume expansion, saline, for supporting cardiovascular function and reducing brain injury.

Maternal low protein diet leads to low podocyte endowment

Cullen-McEwen LA, van der Wolde J, Tribolet L, Puelles VG and Bertram JF

Department of Anatomy and Developmental Biology, School of Biomedical Sciences, Monash University, Melbourne, Victoria, Australia.
john.bertram@monash.edu

Background: Many studies have shown that an adverse feto-maternal environment has a direct effect on renal development. Podocytes are post-mitotic epithelial cells with very limited capacity for regeneration. Given that podocyte loss is a direct cause of glomerulosclerosis, the number of podocytes per glomerulus at the end of nephrogenesis – podocyte endowment – may play a central role in the development of kidney disease. However, whether an adverse feto-maternal environment results in altered podocyte endowment remains unclear.

Aims/Hypothesis: The aim of this study was to determine podocyte endowment in neonatal rats exposed to maternal low protein diet.

Methods: Kidneys were collected at postnatal day 21 in rats exposed to a maternal low (LPD; 8%) or normal (NPD; 20%) protein diet starting at 3 weeks prior to pregnancy, during pregnancy, and until weaning. Total nephron number was estimated by design-based stereology in NPD (n=14) and LPD (n=13) offspring. Podocyte number was determined using a combination of immunofluorescence, confocal microscopy and optical clearing; and glomerular volume was estimated by model-based stereology (n=4 per group). Podocyte density (podocyte number per unit volume of glomerulus) was also calculated.

Results: Body weight, kidney weight and nephron number were 43% ($P<0.0001$), 53% ($P<0.0001$) and 31% ($P<0.0001$) lower in LPD offspring than in NPD offspring. Mean glomerular volume was 45% lower in LPD offspring ($P<0.01$). Interestingly, glomeruli from the LPD group contained 12% fewer podocytes than those from the NPD cohort ($P=0.01$). Consequently, podocyte density was significantly increased in LPD offspring ($P<0.05$).

Conclusions: This is the first report to show that podocyte endowment can be affected by an adverse feto-maternal environment.

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Session 8

Chairs – Julie Owens and Kelly Crossley			
10:00	**A42**	Peter Nathanielsz	The need for nonhuman primate studies to determine mechanisms of developmental programming
10:30	**A43**	Angela Cumberland (L)	The combination of IUGR and prenatal maternal stress changes developmental profiles in guinea pigs
10:42	**A44**	Kirsten McInerney (E)	Altered patterns of behaviour with increasing age in male offspring following perinatal stress
10:54	**A45**	Kathryn Gatford	The metabolic response to exercise is sex-dependent and altered in sheep offspring of placentally-restricted multi-fetal pregnancies
11:06	**A46**	Lara Bush (Hons)	The neuroprotective effects of umbilical cord blood stem cells in intrauterine growth restriction
11:18	**A47**	Mitchell Lock (E)	Expression of miR-133a and miR-15 family and their target genes in the fetus and 6 month old sheep heart in response to myocardial infarction
11:30	General discussion		

The need for nonhuman primate studies to determine mechanisms of developmental programming

Peter Nathanielsz, Cun Li, Matthias Schwab, Geoffrey Clarke, Hilary Huber, Min Du, Kate Keenan, Laura Cox

Texas Pregnancy and Life Course Health Center, San Antonio Texas and Wyoming Life Course Health Center University of Wyoming.
Peter.Nathanielsz@uwyo.edu

Different species have evolved specific responses to nutritional and other challenges by adverse early life events that alter the trajectory of organ development and result in developmental programming. There is much to learn about mechanisms from a comparative study of the responses of each species if we ask the right questions and monitor the correct end points. Most of the studies on developmental programming have been conducted in altrical, polytocous rodents, species with a different growth pattern and developmental endocrine, cardiovascular and reproductive life course trajectory.

Differences between altricial rodents and precocial species are particularly pronounced with regard to the trajectory of development of the hypothalamo-pituitary-adrenal axis (HPAA). Glucocorticoids are central orchestrators of differentiation in preparation for extra uterine life. They are especially important in regulating the balance between proliferation and differentiation during development. There are consequences arising from the difference that the perinatal increase in glucocorticoids in rats and mice occurs post-natally while in precocial special such as humans, sheep and nonhuman primates, the exponential perinatal cortisol rise is prenatal.

We have developed a baboon model of moderate maternal under nutrition (30% reduction in global nutrition) leading to IUGR of about 11% in males and females [1,2]. In this model we have shown changes in placental development [3], accelerated development of the fetal HPAA [4], altered hypothalamic arcuate nucleus feeding thermostat [1], delays in development of the cerebral cortex [5], fetal pancreatic [6], liver [7,8], kidney [2], heart [9,10], and skeletal muscle [11] dysfunction. Postnatally we have shown early emergence of insulin resistance [12] cognitive impairment [13] accompanied by increased aggression [14]. Finally, we have shown accelerated aging of the IUGR offspring cardiovascular system [15] and the brain [16].

This work was supported by NIH P01 HD 21350 and R24 0211367. We would like to thank the many technicians and colleagues who have participated in the very complex team effort. As this is a Festschrift we acknowledge the great friendship over the years of many colleagues who like Richard Harding have made this work so enjoyable. We would like specifically to thank Thomas McDonald.

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The combination of IUGR and prenatal maternal stress changes developmental profiles in guinea pigs

Angela Cumberland[1,2], Hannah Palliser[1,2], Poonam Rani[1,2], Jonathan Hirst[1,2]

[1]School of Biomedical Sciences and Pharmacy, Faculty of Health and Medicine, University of Newcastle, Callaghan, Australia; [2]Mothers and Babies Research Centre, Hunter Medical Research Institute, New Lambton Heights, Australia.
angela.cumberland@uon.edu.au

Background: Intrauterine growth restriction (IUGR) and maternal stress in pregnancy impedes fetal neurodevelopment, with long-term consequences. IUGR is known to impair hippocampal development, which is associated with poorer memory performances whilst prenatal stress can decrease neuronal density. Such deficits are associated with an increased risk of anxiety disorders in later life. Additionally, these insults have been shown to influence neurosteroid levels in the fetus, which may contribute to the adverse neurodevelopmental affects. In this present study, we examined altered neurodevelopmental profiles following IUGR and combined IUGR/stress, and changes in neurosteroid levels.

Aims/Hypothesis: To examine the affects of IUGR and IUGR combined with prenatal stress on fetal development and neurosteroid profiles.

Methods: Dams were allocated to either sham or growth restriction surgery on GA30. IUGR was induced by constricting the cervical and ovarian ends of the uterine arteries with sterile silicon tubing. After recovery, IUGR dams were further allocated to control or stress protocols. Stressing commenced on GA40, for 2 hrs by strobe light, and repeated every 5 days until tissue collection. Fetuses were collected at term (GA69), or on presentation of labour, at which point physical and organ measurements were taken, as well as brain, placental tissue and plasma. IUGR was deemed as a weight <73g, and BLR>0.75.

Results: IUGR/stressed pregnancies displayed signs of labour earlier than sham and IUGR alone pregnancies (p=0.05). All IUGR and IUGR/stressed fetuses were significantly smaller in all physical characteristics than sham fetuses. No differences existed between groups for brain weight. Males from IUGR and IUGR/stressed mothers had significantly less visceral and peripheral fat stores than control males (p=0.0078). IUGR females had lower circulating allopregnanolone compared to control females (p=0.0058), however males did not differ. Neurological outcomes will be assessed.

Conclusions: As expected, IUGR affects all growth parameters, however no additional deficits were caused by prenatal stress. No differences in brain weight with reduced organ weights indicating brain sparing occurred, whilst males appeared to sacrifice fat reserves more compared to females. IUGR females have lower allopregnanolone, however IUGR/stressed females did not show the same reductions. Longer term affects on neurological outcome will be determined.

Altered patterns of behaviour with increasing age in male offspring following perinatal stress

McInerney, Kirsten C[1, 2], Palliser, Hannah K.[1, 2], Hirst, Jonathan J[1, 2]

[1]School of Biomedical Science and Pharmacy, University of Newcastle, Newcastle, New South Wales, Australia, [2] Mothers and Babies Research Centre, Hunter Medical Research Institute, Newcastle, New South Wales, Australia.
kirsten.mcinerney@uon.edu.au

Background: Both prenatal stress and maternal separation have been associated with detrimental outcomes, including behavioral pathologies such as increased anxiety in offspring. This study aimed to determine the effect of combined prenatal and maternal separation stress on guinea pig offspring behaviour at the equivalent ages of childhood and adolescence.

Methods: Guinea pig dams were exposed to strobe light for 2 h on gestational days 50, 55, 60 and 65. They were allowed to deliver naturally. Pups were subjected to a second stress of separation from dams for 2 h daily from postnatal day 2 – 8 (Dual stress exposure). All pups then underwent behavioural evaluation using the Elevated Plus Maze (EPM), Open Field (OF) and Acoustic startle testing at 8 and 28 postnatal days of age (PND) before tissue collection at PND30.

Results: At PND8, the equivalent of childhood in the guinea pig, male offspring exposed to the dual stress displayed more entries in to the closed arms of the EPM as well as spent more time in these arms indicating higher levels of anxiety. At PND28 dual stress male offspring spent more time in the open arms of the EPM, as well as travelled further distances in the OF indicating greater levels of hyperactivity. Acoustic startle indicated a decrease in prepulse inhibition with stress.

Conclusions: Perinatal stress causes male offspring to exhibit anxiety like behaviours in childhood, transitioning to hyperactivity-like behaviors in adolescence highlighting the effect of aging on the behaviour of offspring that were exposed to perinatal stresses.

The metabolic response to exercise is sex-dependent and altered in sheep offspring of placentally-restricted multi-fetal pregnancies

Kathryn L Gatford[1,2], Amy L Wooldridge[1,2], Manpreet Kaur[1,2], Jennifer Rice[1], Anna Le Ber[2], Filippe Falcao-Tebas[3], Jarrod Kerris[3], Evelyn Marin[3], Glenn K McConell[3]

[1]Robinson Research Institute and [2]School of Medicine, University of Adelaide; [3]Institute of Sport, Exercise and Active Living, Victoria University, Australia.
kathy.gatford@adelaide.edu.au

Background: Exercise training improves glucose homeostasis, improving insulin sensitivity and secretion, which are both impaired after intrauterine growth restriction (IUGR). There is limited evidence that responses to exercise are blunted after IUGR, but this has not been tested in adult humans or animals after IUGR nor mechanisms identified.

Aims/Hypothesis: To measure the metabolic responses to adult exercise training in progeny of control (CON) and placentally-restricted (PR) ovine pregnancy.

Methods: Glucose tolerance and insulin secretion were measured during an intravenous glucose tolerance test in one year-old (adult) progeny from multi-fetal CON (n=5 M, 9 F) and PR (n=9 M, 10 F) pregnancies. Sheep were re-tested after 33 d of exercise training (~3.4 km running each day in same-sex groups, average speed 5.7 ± 0.06 km/h). Effects of training, progeny treatment, and progeny sex were analysed using a repeated measures model, including the dam as a random factor to account for maternal environment in twins.

Results: Before training, fasting glucose was ~7% higher in PR than CON progeny, but glucose tolerance and insulin secretion did not differ between groups. Training profoundly reduced the increase in circulating lactate after daily exercise, similarly in all animals. The effect of training on fasting glucose differed between treatments and sexes, decreasing fasting glucose in PR overall ($P=0.005$) but not CON, and in females overall ($P<0.001$), and within CON ($P=0.045$) and PR ($P=0.004$) females, but not in males. Glucose tolerance improved by ~10% after training in CON ($P=0.028$) but did not change in PR progeny. The insulin secretion response to glucose increased >40% overall after training ($P=0.009$), and this training response occurred in CON ($P=0.005$) but not PR animals when analysed separately.

Conclusions: Exercise training improves markers of metabolic health differently in CON and PR sheep. Enhanced insulin secretion appears to underlie training-induced improvements in glucose tolerance in CON but not PR progeny; our preliminary analyses indicate that this reflects improved pancreatic function not loss of insulin sensitivity. Our results suggest that individuals subjected to a restricted environment before birth may have impaired capacity to respond to adult exercise. We hypothesise that interventions to reprogram metabolism and reverse or prevent adverse metabolic effects of IUGR that develop with aging may need to be targeted to earlier ages, during periods of greater plasticity.

The neuroprotective effects of umbilical cord blood stem cells in intrauterine growth restriction

Bush L[1], McDonald C[1], Sutherland A[1], Mihelakis J[1], Malhotra A[1,3], Fahey M[1,3], Jenkin G[1,2], Miller S[1,2], Castillo-Melendez M[1]

[1]The Ritchie Centre, Hudson Institute of Medical Research, Clayton, Australia,[2]Dept Obstetrics & Gynaecology, Monash Medical Centre, Clayton, Australia, [3]Monash Newborn, Monash University, Clayton, Australia.

lebus2@student.monash.edu

Background: Intrauterine growth restriction (IUGR) is a fetal disease that is associated with stillbirth, preterm delivery and poor neurodevelopmental outcomes. Despite affecting 5-10% of pregnancies worldwide, there is currently no standard treatment for IUGR. In animal models, IUGR has been shown to damage to the structure of the brain, with disorganisation of grey matter and alterations to the cerebrovasculature. We investigated the effects of umbilical cord blood (UCB) stem cells, a novel therapy, on the cerebrovasculature and grey matter of fetal ovine, IUGR brains.

Aims/Hypothesis: Our aim was to determine if UCB stem cells could improve the structure of the brain in a fetal, ovine model of IUGR. We hypothesised that the UCB stem cells would have reparative effects by increasing neuron numbers, decreasing cell death and restoring the integrity of the cerebrovasculature within the brain.

Methods: For this study we utilised the single umbilical artery ligation (SUAL) ovine model to produce placental insufficiency, and thus growth restriction in our fetuses. Ewes pregnant with twins underwent the SUAL procedure for one fetus, with the other fetus acting as a control. Healthy, term ovine UCB cells were administered to the fetuses at 125 days gestation. Post-mortems was performed at 135 days gestation and the fetal brains were collected. The groups studied were: control (n=6), IUGR (n=5), control&cells (n=3) and IUGR&cells (n=3). To analyse the cerebrovasculature structure, one brain from each group was infused with fluorescent dextrans. The numbers of apoptotic cells (Caspase-3) and neurons (NeuN) within the brain were also analysed.

Results: The dextrans showed an inherent leakiness in the IUGR brains compared to control brains, this leakiness show reparation with UCB stem cell administration as it was not present in the IUGR&cells group. Apoptotic cell death (Caspase-3) was increased in the IUGR cortex ($p<.05$) when compared to control. However, there was no difference in the number of neurons in the cortex (NeuN) between groups. The IUGR&cells group showed a trend towards further increased cell death within the cortex but also increased neurons.

Conclusions: The administration of UCB cells reduced the leakiness of the cerebrovasculature and encouraged neuron growth of the grey regions within the brain, despite further increasing apoptotic cells. Additional analysis of the cerebrovasculature and grey matter with increased animal numbers will add to this preliminary research.

Expression of miR-133a and miR-15 family and their target genes in the fetus and 6 month old sheep heart in response to myocardial infarction

Mitchell Lock[1], Jia Yin Soo[1], Jack Darby[1], Doug Brooks[2], Enzo Porrello[3], Ross Tellam[4], Janna Morrison[1]

[1]Early Origins of Adult Health Research Group and [2]Mechanisms in Cell Biology and Disease Research Group, Sansom Institute for Health Research, University of South Australia; [3]Centre for Cardiac and Vascular Biology, School of Biomedical Sciences, University of Queensland;. [4]CSIRO Agriculture, Queensland Biosciences Precinct.
mitchell.lock@mymail.unisa.edu.au

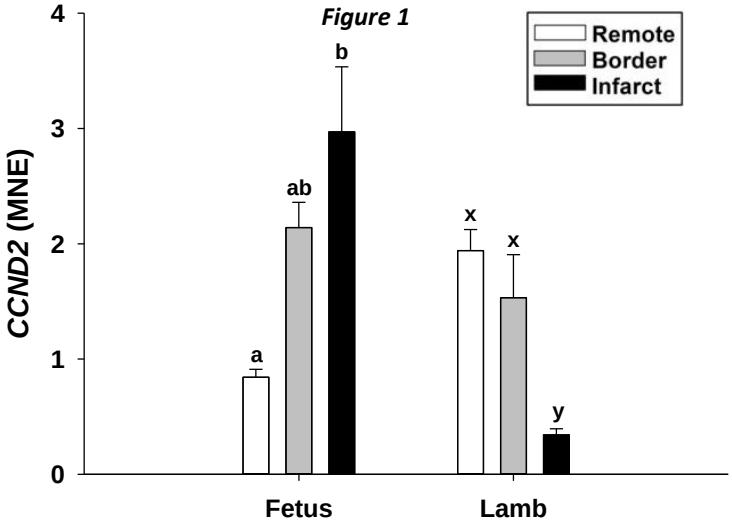
Background: The adult mammalian heart has little capacity to repair after damage because adult cardiomyocytes lack the ability to proliferate. This lack of response to injury is in stark contrast to the adult zebrafish, newborn mouse and fetal sheep whose cardiomyocytes have the capacity to repair through the proliferation of existing cardiomyocytes. The expression of microRNAs (miRNAs) such as miR-133a and the miR-15 family increase at the time that proliferation ceases in rodents and sheep and have been implicated in regulating cell cycle entry and cardiomyocyte proliferation.

Aims/Hypothesis: We aimed to investigate the expression of these miRNAs and their target genes after myocardial infarction in sheep hearts before (105 d gestation) and after (6 months) birth when the response to injury is different.

Methods: We used sheep, which have similar cardiomyocyte development to humans, as a model to investigate the effect of age on cardiac damage, by ligating the second diagonal of the left anterior descending (LAD) coronary artery. Surgery was performed on fetuses (102 days gestation when all cardiomyocytes are proliferative) and postnatal sheep (6 months of age when all cardiomyocytes contribute to heart growth by hypertrophy). Three days later, infarct size was visualized using 2,3,5-triphenyltetrazolium chloride (TTC) staining of heart sections. Total RNA was extracted and qRT-PCR was used to quantify expression of miRNA and their target genes.

Results: Mean normalized expression (MNE) of target genes of miR-133a (e.g. *SRF* and *IGF1R*) and the miR-15 family (e.g. *CCND2* (Figure 1)) were increased in myocardial tissue from the infarcted area compared with the remote zone in the fetus. In contrast, the opposite expression pattern was observed in the lamb.

Figure 1



Conclusions: These results indicate that miR-133a and the miR-15 family have significant roles in regulating cardiomyocyte proliferation in sheep. Low expression in the fetus allows cardiac repair after damage with high expression after birth resulting in limited capacity for repair.

Session 9

Chairs – Frank Bloomfield and David Todd			
12:15	**A48**	Stuart Hooper	Non-invasive ventilation in the delivery room
12:35	**A49**	Marcus Davey	Extrauterine support for extreme prematurity
12:47	**A50**	Fiona Stenning (E)	Effects of oxytocin administration during physiological-based cord clamping on the cardiorespiratory transition at birth
12:59	**A51**	Joseph Smolich	Blunted sympathoadrenal activation with increased haemodynamic stability at birth in preterm lambs: immediate & delayed cord clamping
13:11	**A52**	Zeena Al-Obaidi (H)	Evaluating the relationship between diaphragm function and respiratory failure in preterm infants
13:23	General discussion		

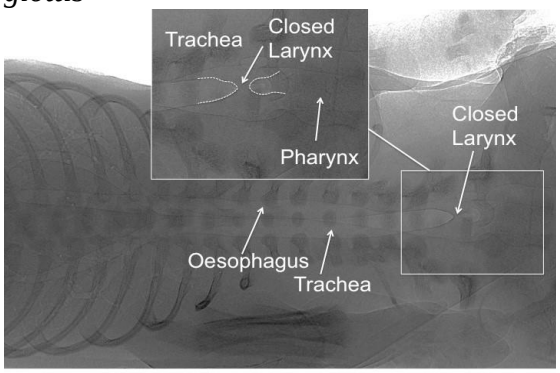
Non-invasive ventilation in the delivery room

Stuart B Hooper[1,2] Jessica Crawshaw[1,2], Marcus J Kitchen[3] Megan J Wallace[1,2] Katie Lee[3], Marta Thio[4], Peter G. Davis[4] and Arjan B. te Pas[5]

[1]The Ritchie Centre, Hudson Institute of Medical Research, Melbourne, Australia; [2]Department of Obstetrics and Gynaecology and [3]School of Physics, Monash University, Melbourne, Australia; [4]Royal Women's Hospital, Melbourne, Australia, and [5]Neonatology, Leiden University Medical Centre, Leiden The Netherlands.
stuart.hooper@monash.edu

Aeration of the lung at birth underpins the transition to newborn life. However, many infants born premature are unable to adequately aerate their lungs and need respiratory support to survive. But, as they are immature, preterm infants are highly vulnerable to lung and brain injury, which has severe long-term consequences. As such much effort has focused on protecting the lung and brain from injury leading to the widespread use of non-invasive ventilation in the delivery room, usually applied via a facemask. This includes intermittent positive pressure ventilation (iPPV) or the application of continuous positive airway pressure (CPAP, usually 5cmH_2O) to support spontaneous breathing.

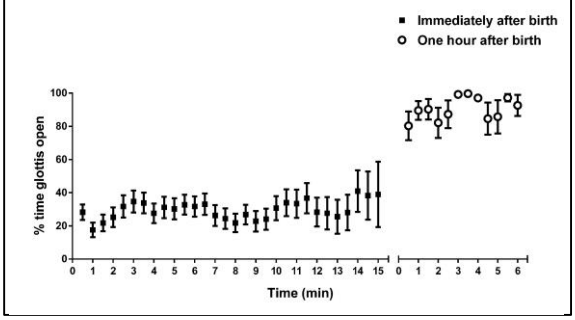
Fig 1. Phase contrast X-ray image of a newborn preterm rabbit with a closed glottis



While non-invasive ventilation is now used world-wide, little or no information is available on how it interacts and integrates with the infant's changing physiology at birth. It is not known if the strategies applied are effective, counterproductive or even injurious, but despite this they have been universally adopted as the first choice for respiratory support at birth.

Using phase contrast X-ray imaging, we have imaged the larynx at birth in spontaneously breathing preterm rabbits that were supported with non-invasive ventilation (Fig 1). We found that the larynx is mostly closed at birth and opens only briefly during inspiration (Fig 2). By adducting the vocal cords the larynx seals the trachea and prevents airflow, making ventilation using a facemask ineffective unless the infant opens its vocal cords (glottis) to breathe. However, within an hour of birth we found that the glottis was mostly open and that the lungs could be ventilated non-invasively (Fig 2). This indicates that laryngeal function switches after birth, from a state where it is mostly closed into a state where it is mostly open.

Fig. 2 Percentage of time larynx (glottis) is open immediately after birth



In conclusion, we found that at birth the glottis is mostly closed, which prevents non-invasive ventilation of the lung. As such, the stimulation of spontaneous breathing is an essential component of non-invasive respiratory support when applied immediately after birth. More research is required to understand the mechanisms that control the switch in glottis function after birth.

Extrauterine support for extreme prematurity

MG Davey, EA Partridge, MA Hornick ,JD Vrecenak, C Mesas-Burgos, A Olive, RC Caskey, TR Weiland, J Han, JT Connelly, KC Dysart, J Rychik, HL Hedrick[1], WH Peranteau, AW Flake

The Center for Fetal Research, The Children's Hospital of Philadelphia Research Institute, 3615 Civic Center Blvd., Philadelphia, 19104, USA.
daveym@email.chop.edu

Background: Extreme prematurity is the leading cause of neonatal mortality and morbidity due to organ immaturity and iatrogenic injury. Despite considerable advances in neonatal care, the prospect of a significant improvement in outcome seems limited with current management strategies. The development of a physiologic extrauterine system to support normal fetal development would abrogate the consequences of prematurity and fundamentally change the way preterm infants are managed. Since the 1950's, significant efforts to achieve prolonged extrauterine life support have met with limited success primarily due to inability to recapitulate key aspects of normal fetal physiology. We developed an extracorporeal support system (ESS) to promote physiologic development by maintaining fetal sheep in a sterile fluid environment and providing umbilical gas exchange via *pumpless* arteriovenous (A-V) ECMO.

Aim: Develop a *pumpless* ESS to provide prolonged (up to 4 weeks) physiologic support of preterm fetal sheep.

Methods/Results: Briefly, preterm lambs were delivered by caesarean delivery, connected to the ESS, and placed in a temperature-regulated fluidic incubator. We reasoned that a pumpless circuit, in which blood flow is driven exclusively by the fetal heart, combined with a low resistance/volume oxygenator (Neonatal Quadrox, Maquet, Germany) and short segments of tubing would most closely mimic the normal fetal/placental circulation. Initially, the carotid artery (CA) and jugular vein (JV) were used for A-V access in late-gestation lambs (120-140d GA) resulting in remarkable hemodynamic stability during runs (23 to 108 hours). A major limiting factor in pilot studies was sepsis related to the fluid environment. A semi-closed glass tank with continuous fluid exchange was designed and allowed longer studies (346.6 +/- 93.5 hours); importantly, one animal was maintained on the circuit for 288 hours (120 – 132 days gestation) and weaned to spontaneous respiration with long-term survival. To further address issues of sterility, size adaptability, and efficiencies of space and fluid volume, a "Biobag" design was developed which essentially solved the problem of gross fluid contamination, and eliminated severe pneumonia on lung pathology. With increasingly younger fetuses, CA/JV cannulation resulted in high-pressure venous return via the superior vena cava. To offload the right heart, we utilized a CA-umbilical vein (UV) cannulation strategy (N=5; 106-113d GA) supporting lambs 13 to 26 days in the Biobag. However, flow to the oxygenator in CA/UV lambs was well below normal placental flow (70–120 vs. 150-200 ml/kg/min) primarily due to limited inflow from the small calibre CA. To improve circuit flow, a double umbilical artery (UA)/UV strategy was developed and enabled the study of 105-108 fetuses for up to 25-28 days. Circuit flow for UA/UV lambs (150-200 ml/kg/min) was comparable to in vivo levels throughout runs resulting in normal oxygen delivery with fetal levels of A-V oxygen saturation. UA/UV animals had normal pH values and lactate levels throughout runs. Daily echocardiography confirmed physiologic cardiac outputs and maintenance of the fetal cardiac circulation with near-normal ductus arteriosus flows, and right-to-left shunting through the foramen ovale. Pulmonary morphometric analysis of UA/UV lambs demonstrated maturation from the canalicular to saccular/alveolar stages of lung development with normal density of alveolar epithelial type-2 cell densities. Functionally, lambs were easily ventilated after removal from the circuit, and nearly comparable to age-matched control lambs.

Conclusions: The pumpless ESS system represents a potential strategy to abrogate the pulmonary consequences of extreme prematurity and will provide new experimental insight into placental function.

Effects of oxytocin administration during physiological-based cord clamping on the cardiorespiratory transition at birth

Fiona Stenning[1], Graeme Polglase[1], Valerie Zahra[1], Alison Moxham[1], Kelly Crossley[1], Domenic LaRosa[1], Euan Wallace[1], Karyn Rodgers[1], Martin Kluckow[2], Andy Gill[3] and Stuart Hooper[1]

[1]The Ritchie Centre, The Hudson Institute of Medical Research and Department of Obstetrics & Gynaecology, Monash University, Clayton, VIC, Australia; [2]Department of Neonatology, Royal North Shore Hospital, University of Sydney, Sydney, NSW, Australia; [3] Centre for Neonatal Research and Education, University of Western Australia, Subiaco, WA, Australia.
fiona.stenning@monash.edu

Background: Delaying umbilical cord clamping (UCC) until ventilation has been established (physiological-based cord clamping; PBCC) improves the cardiovascular transition at birth in preterm neonates. In this setting the influence of oxytocin (*Syntocinon)*, usually administered to the mother at delivery of the anterior shoulder of the fetus to prevent post partum haemorrhage (PPH), is unknown.

Aims/Hypothesis: We aimed to determine the effect of standard oxytocin administration on neonatal oxygen saturation during the cardiopulmonary transition at birth in preterm lambs and subsequently, term infants.

Methods: Preterm sheep fetuses (0.83 gestation) were exposed by caesarean section and instrumented for recording of Umbilical arterial (UAF) and venous flow (UVF), carotid arterial pressure (CaP) and flow (CaF), and pulmonary blood flow (PBF). Arterial oxygen saturation (SpO_2) and cerebral oxygenation ($SctO_2$) were continuously measured. Ewes were administered 10 IU oxytocin (n=5) or saline (control, n=5), lambs were delivered and ventilation was initiated ~ 15 min prior to, and for ~15 min after, umbilical cord clamping.

Results: Oxytocin caused vigorous uterine contractions, resulting in a rapid decrease in umbilical arterial (by 41.7 $\pm$ 21.8%) and venous flow (by 41.0 $\pm$ 29.8%) and an increase in CaF (by 22.0 $\pm$ 13.1%) and heart rate (by 39.8 $\pm$ 23 bpm) in the first 3 min compared to controls. Uterine contractions caused arterial oxygen saturation and cerebral oxygenation to decrease by 12.3 $\pm$ 11.8% and 27.9 $\pm$ 19.2% respectively. Ventilation restored SpO_2 and $SctO_2$ in the oxytocin lambs, but CaP and HR remained higher and umbilical flow remained lower in the oxytocin lambs.

Conclusions: Early oxytocin administration negates the benefits of delayed UCC. Given that oxytocin can be safely administered after UCC without increasing the risk of PPH, our findings suggest that delaying oxytocin administration until after UCC in the setting of delayed UCC may be of benefit. We are currently undertaking a RCT at Monash Health assessing the effect of oxytocin administration during delayed cord clamping on neonatal oxygen saturation after birth in term infants.

Blunted sympathoadrenal activation with increased haemodynamic stability at birth in preterm lambs: immediate & delayed cord clamping

Joseph J. Smolich[1], Kelly R. Kenna[1], Sarah E. Phillips[2], Gavin W. Lambert[2]

[1]Heart Research Group, Murdoch Childrens Research Institute, Parkville, Victoria, Australia;
[2]Human Neurotransmitters Laboratory, Baker-IDI, Prahran, Victoria, Australia.
joe.smolich@mcri.edu.au

Background: Perinatal haemodynamic fluctuations after immediate cord clamping (ICC) and an extended (1.5-2min) pre-ventilation interval closely resemble changes seen with in utero asphyxia, which is accompanied by striking rises in plasma levels of the catecholamines noradrenaline and adrenaline. By contrast, only minor haemodynamic fluctuations occur with ICC and a brief (0.5min), non-asphyxial pre-ventilation interval, or delayed cord clamping (DCC), implying that perinatal rises in plasma catecholamines (and thus sympathoadrenal activation) are attenuated with the latter delivery strategies.

Hypothesis: A surge in circulating catecholamines accompanies perinatal haemodynamic fluctuations after ICC with an extended pre-ventilation interval, but this surge is blunted after either ICC and a brief pre-ventilation interval, or DCC.

Methods: Anaesthetized preterm fetal lambs were instrumented at 128±1 days with 1) brachiocephalic trunk and aortic isthmus flow probes to measure left ventricular (LV) output, 2) ductal and left pulmonary artery flow probes to measure right ventricular (RV) output, 3) aortic trunk (AoT) catheters to measure pressures and to obtain samples for blood gas analysis and plasma catecholamine assay (via HPLC). While haemodynamics were recorded continuously, fetuses were delivered onto the ewe's abdomen and the cord clamped immediately (ICC) for 1.5min (n=9) or 0.5min (n=5) prior to mechanical ventilation, or ventilated for 1.5min prior to cord clamping (DCC, n=6). AoT sampling for blood gas analysis and catecholamine assay was performed after initial cord clamping or ventilation, and then regularly after birth.

Results: With ICC for 1.5min, an asphyxial state (pH 7.25, Po_2<10mmHg, Pco_2>56mmHg) was evident by 1min, with falls in LV and RV outputs (by 50% & 32%, P<0.001), followed by a transient tachycardia with surges in LV and RV outputs (4- & 2.4-fold, P<0.001) and blood pressure after ventilation. By contrast, perinatal haemodynamics were relatively stable after ICC for 0.5min, or DCC. Baseline fetal noradrenaline and adrenaline levels were similar in the 3 groups (average 208±78 and 26±27 pg/ml respectively). With ICC for 1.5min, plasma noradrenaline and adrenaline rose 10- and 47-fold respectively (P<0.001), and declined over 5min after birth. By contrast, plasma noradrenaline rose only 77-90% (P<0.01) and adrenaline 4.4 to 8.5-fold (P<0.005) in the perinatal period with ICC and a brief pre-ventilation interval, or DCC.

Conclusions: Perinatal haemodynamic fluctuations after ICC with an extended (i.e. asphyxial) pre-ventilation interval are accompanied by a substantial sympathoadrenal activation. Such activation is markedly blunted in the setting of increased haemodynamic stability seen with ICC and a brief (i.e. non-asphyxial) pre-ventilation interval, or DCC.

Evaluating the relationship between diaphragm function and respiratory failure in preterm infants

Zeena Al-Obaidi[1,2], Jane Pillow[1,2], Gavin Pinniger[1], Ben Stoecklin[2]

[1]School of Anatomy, Physiology and Human Biology and [2]Centre for Neonatal Research and Education, University of Western Australia, 35 Stirling Highway, Perth, WA 6009, Australia.
21127637@student.uwa.edu.au

Background: Preterm birth is the leading cause of perinatal morbidities, the most prevalent being respiratory failure. The diaphragm is the most important respiratory muscle and an active diaphragm is pivotal for spontaneous breathing from birth. The structural and functional immaturity of the preterm diaphragm, combined with the increased work of breathing in neonates, is likely to contribute to respiratory failure in preterm infants. However, the relationship between diaphragm function and risk factors for respiratory failure in preterm infants is explored in few studies.

Aims/Hypothesis: This study aimed to identify the associations between diaphragm function and risk factors for respiratory failure in preterm infants: maturity at birth, the duration of mechanical ventilation, and fetal growth. We hypothesised that maturity at birth and the duration of mechanical ventilation would independently predict diaphragm function.

Methods: Measurements were performed on 121 very preterm infants at approximately 36 weeks postmenstrual age. Diaphragm function (force and work) was derived from transdiaphragmatic pressure measurements recorded during tidal breathing. Transdiaphragmatic pressure was calculated from simultaneous measurement of oesophageal and gastric pressures using a dual pressure transducer (2 F Mikro-tip). The associations between diaphragm function and risk factors for respiratory failure, as well as collinear variables, were identified using bivariate regression analysis. Multivariate regression analysis was then performed to identify independent predictors of diaphragm function.

Results: Bivariate analysis indicated that both diaphragm force and work correlated significantly with various maturity factors, and growth factors. Collinearities were identified between the maturity and growth factors: only the most significant maturity factor and growth factor were entered into the multivariate analysis model. The multivariate regression analysis indicated that maturity at birth is the most significant predictor of diaphragm force ($P = 0.003$) and work ($P = 0.002$). Fetal growth also independently predicts diaphragm force ($P = 0.014$) and work ($P = 0.039$), but to a lesser extent than maturity at birth.

Conclusions: Preliminary analyses suggest that two of the major risk factors for developing respiratory failure in preterm infants, namely maturity at birth and fetal growth, independently predict diaphragm function. It is therefore possible that diaphragm function contributes to the development of respiratory failure in very preterm infants.

Funding: Loman-Hall Scholarship (ZA-O); NHMRC CRE 1057514; NHMRC PG 1047689; NHMRC Fellowship 1077691 (JP)

Session 10

Chairs – Tamás Zakár and Ian Wright			
2:30	**A53**	Megan Wallace	Identifying the factors that regulate lung development
2:50	**A54**	Emily Cohen (L)	Growth restricted preterm neonates display compromised heart rate variability on the first postnatal day
3:02	***A55**	Keiji Suzuki	Ionized and total magnesium in the plasma in neonates - in comparison with calcium
3:09	***A56**	Donna Rudd	The effect of body weight on serum creatinine and cystatin C measurements in neonates
3.16	***A57**	Christiane Theda	GABA Receptor 1 gene methylation changes as an example of differential DNA methylation in blood of preterm versus term babies
3:23	General discussion		

Identifying the factors that regulate lung development

Megan Wallace

The Ritchie Centre, Hudson Institute of Medical Research and The Department of Obstetrics and Gynaecology, Monash University, Clayton, Vic. Australia.
megan.wallace@monash.edu

Background: Survival at the time of birth is critically dependent on the lungs being adequately developed. If the lungs have not reached an adequate stage of development by the time of birth, respiratory support may be required, which can injure the lungs and lead to abnormal lung development. In this talk, Megan will outline the studies that she and her group have used to identify candidate genes involved in normal and abnormal lung development. There will be a particular focus on the genes Trop2, CTGF, Cyr61 and EGR1.

Growth restricted preterm neonates display compromised heart rate variability on the first postnatal day

Emily Cohen[1,2,4], Alexsandria Odoi[1], Samantha Hollis[1], Joanne Mockler[1,3], Flora Wong[1,2], Euan Wallace[1,3], Rosemary Horne[1,2], Stephanie Yiallourou[1,2]

[1]The Ritchie Centre, Hudson Institute of Medical Research, [2]Department of Paediatrics, Monash University, [3]Department of Obstetrics and Gynaecology, Monash University, Melbourne, Australia, [4]Department of Neonatology, University Medical Centre Utrecht, Utrecht, The Netherlands.
emily.cohen@monash.edu

Background: Intrauterine growth restriction (IUGR) is associated with an increased risk of Sudden Infant Death Syndrome (SIDS) and cardiovascular disease in adulthood. SIDS has been linked to compromised autonomic cardiovascular control and cardiovascular disease to sympathetic overactivity. The increased risk for both conditions for IUGR individuals may be due to altered development of autonomic cardiovascular control related to the unfavourable intrauterine environment.

Aims/Hypothesis: To investigate the effects of IUGR on postnatal autonomic cardiovascular control by the means of heart rate variability (HRV) analysis. We hypothesised that IUGR neonates would display compromised HRV with relative sympathetic overactivity.

Methods: 19 preterm IUGR and 17 preterm appropriate for gestational age (AGA) neonates were studied during sleep in the supine position within the first 24h of life. Spectral analysis of the ECG was performed in the low (LF, 0.04-0.15Hz, reflecting sympathetic and parasympathetic activation) and high (HF, 0.4-1.5Hz, reflecting parasympathetic activation) frequency ranges. Total power (TP) and LF/HF ratio (to investigate sympathovagal balance) were also explored. Data were analysed separately for the infant sleep states active sleep (AS) and quiet sleep (QS) and differences between the groups were explored using Student's T-test or Mann-Whitney U analyses as appropriate.

Results: Gestational age and postnatal age at time of study were not different between IUGR and AGA neonates. IUGR neonates had significantly lower birthweights than their AGA peers (1100g ± 365 vs 1657g ± 478, $p<0.001$). Heart rate as derived from the ECG R-R interval was higher in IUGR than AGA neonates in AS (139bpm ± 1 vs 130bpm ± 2, $p<0.05$) and tended to be higher in QS (136bpm ± 1 vs 128bpm ± 2, $p=0.07$). IUGR neonates exhibited reduced HF in AS and reduced LF and TP in both sleep states compared to AGA controls ($p<0.05$ for all). The LF/HF ratio was not different between the groups in either sleep state.

Conclusions: IUGR neonates display compromised HRV on the first postnatal day, which may suggest increased vulnerability to circulatory instability and may predispose these infants to SIDS. In contrast to our hypothesis, IUGR neonates did not demonstrate sympathetic overactivity. Further longitudinal studies within this cohort will examine how autonomic cardiovascular control develops throughout infancy.

Ionized and total magnesium in the plasma in neonates - in comparison with calcium

Keiji Suzuki

Department of Paediatrics, Tokai University School of Medicine, 143 Shimokasuya, Isehara, Kanagawa 259-1193, Japan.
dkswnd@tokai-u.jp

Background: Magnesium (Mg) exists in the plasma 55-70% as ionized form and the rest bound to proteins and organic anions. Status of Mg in the plasma of the newborn is not well understood.

Aims: To study ionized and total magnesium (iMg and tMg) in the plasma in neonates in the NICU in comparison with ionized and total calcium (iCa and tCa) .

Methods: Subjects are term and preterm newborn infants admitted to the NICU of Tokai University Hospital in Jun-Jul 2012. Blood samples were taken either within 24 hrs of birth (early) or beyond 1 week of birth (late). Plasma iMg and iCa were measured by selective ion electrode method using Stat Profile pHOx Ultra® (Nova Biomedical Co.) and plasma tMg was measured by OCPC method using SpotChem II Mg® (Arkray. Co.). Plasma tCa was measured by methyl-xylenol-blue method. The values of iMg and iCa were adjusted for pH for analysis. Temporal changes and correlations between iMg, tMg, iCa and tCa were studied.

Results: Fourteen early (<24hr) samples (0-18 hrs, GA 26-40wk, BW 910-3355gr) and 7 late (>1wk) samples (day11-66, GA 30-39wk, BW 1750-2812gr) were studied. iMg and tMg were high but iCa and tCa were low within 24 hrs of birth, all of which normalized over 7 days. The %change at birth was less in iMg than in tMg but more in iCa than in tCa.

parameter	< 24 hrs	>7 days
iMg (mmol/L)	0.58±0.05	0.52±0.01
iCa (mmol/L)	1.03±0.05	1.27±0.02
tMg (mmol/L)	1.23±0.17	0.86±0.05
tCa (mmol/L)	2.24±0.05	2.40±0.05

Data represented as mean±SE

Conclusions: The relative change in ionized fraction was more in Ca and less in Mg. We speculate it could be due to difference in capacity of buffers involved, mobilization of ions between intra- and extra-cellular compartments, renal excretion or gastrointestinal absorption.

The effect of body weight on serum creatinine and cystatin C measurements in neonates

Yogavijayan Kandasamy[1], Donna Rudd[2]

[1]Department of Neonatology, The Townsville Hospital, Queensland 4814, Australia, College of Medicine and Dentistry 2 The James Cook University, QLD 4814 , College of Public Health, Medical and Veterinary Sciences, The James Cook University, QLD 4814, Australia.
Donna.rudd@jcu.edu.au

Background: Serum creatinine measurement has been used to determine glomerular filtration rate (GFR) in both adults, children and neonates. However, creatinine measurement is associated with many pitfalls. Serum creatinine level only starts to rise once the GFR falls below 50%, dependant on muscle mass and in babies, levels in the first few days of life reflect maternal serum creatinine level. Cystatin C is increasing being recognised as a more reliable biomarker of renal function.

Methods: The data presented here is preliminary data from ongoing larger kidney study in neonates. Babies, both term and preterm underwent blood test after 37 weeks of gestation. Serum creatinine and Cystatin C was determined at the same time and its correlation with the babies weight was determined. The babies also underwent renal ultrasound

Results: In this interim analysis, data from 38 neonates are presented. The gestational age for this babies are between 37 to 38 weeks, with weight of 2541 g ± 684 g. The median serum creatinine level is 20.4 [0.9-80] umol/L. The mean Cystatin C level is 1.7±0.2 umol/L. Serum creatinine level has significant correlation with body weight (r=0.4, p =0.02) where as Cystat50in C level had no correlation with body weight (r =0.1, p=0.48). None of the babies has renal failure or congenital renal abnormalities.

Conclusions: Serum creatinine is unreliable biomarker to determine renal function in neonates. Cystatin C is a more reliable option.

GABA Receptor 1 gene methylation changes as an example of differential DNA methylation in blood of preterm versus term babies

Christiane Theda

Royal Women's Hospital, Neonatal Services, Melbourne VIC Australia, Murdoch Childrens Research Institute, Melbourne VIC Australia and University of Melbourne, Melbourne VIC Australia.
Christiane.Theda@thewomens.org.au

Background: Preterm infants, especially those born extremely preterm, are at high risk for long term adverse health effects. Events before and around the time of birth can have persistent biological effects. Increasing evidence links epigenetics to a developmental origin of health and disease. DNA methylation is one epigenetic mechanisms and DNA methylation changes have been reported in blood of preterm versus term babies.

Aim: Review DNA methylation changes found in probes relating to the GABA receptor 1 (GABBR1) gene to demonstrate the utility of DNA methylation array analysis to identify and examine genes of biological relevance in neonatal research.

Methods: The Illumina Infinium HumanMethylation450 BeadChip Array was used to explore genome wide methylation of whole blood spot derived genomic DNA from preterm and term babies in several studies, including two in which the author has participated. Data of DNA methylation of GABBR1 related sites on the array (185 total) were obtained from the author's own publications and research as well as from publications (and related data in the GEO database) reporting on DNA methylation of cord or newborn blood of preterm and/or term newborns.

Results: Most GABBR1 gene related probes are highly methylated at birth. Comparing preterm and term babies at birth we found six GABBR1 probes differentially methylated (DMPs) with preterm probands showing higher methylation levels than term probands (Genome Medicine 2013: 5: 96). Our recent small pilot study investigating early methylation changes in whole blood derived DNA of preterm babies (EpiPrem Study) found that two GABBR1 probes were among 2001 probes showing differential methylation only 7 days after birth. Fernando et al. (BMC Genomic 2015 16:736) report 10 DMPs in GABBR1 in cord blood DNA comparing preterm and term babies. GABBR1 related probes are not among a subset of 168 probes which are highly correlating with gestational age (A. Smith, unpublished), thus changes are unlikely to be purely developmental. Nissen et al recently (Frontiers in Psychiatry, 2016: 7, 35) reported methylation differences in two GABBR1 probes in blood spot derived DNA of newborns who later developed obsessive-compulsive disorder.

Conclusions: While the biological significance of the reported methylation changes in GABBR1 remains to be shown, the author hopes the example given will demonstrate to the workshop participants the potential utility of genome wide DNA methylation studies in newborn research; the author encourages workshop participants interested in methylation status of genes and molecules of interest to them to make contact with the author and her collaborators.

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Delegates

Dr. Beth Allison *Hudson Institute* *Melbourne, VIC* *beth.allison@hudson.org.au*	**Ms. Zeena Al-Obaidi** *The University of Western Australia* *Perth, WA* *21127637@student.uwa.edu.au*	**Dr. Ali Hani** *Monash University* *Melbourne, VIC* *ali.hani@monash.edu*
Ms. Christine Astell *The University of Western Australia* *Perth, WA* *20496493@student.uwa.edu.au*	**Ms. Jade Barbuto** *Hudson Institute* *Melbourne, VIC* *jade.barbuto@monash.edu*	**Miss. Nadia Bellofiore** *Hudson Institute* *Melbourne, VIC* *nbel10@student.monash.edu*
Prof. Laura Bennet *The University of Auckland* *Auckland, New Zealand* *l.bennet@auckland.ac.nz*	**Prof. John Bertram** *Monash University* *Melbourne, VIC* *john.bertram@monash.edu*	**Dr. Tracey Björkman** *The University of Queensland* *Brisbane, QLD* *t.bjorkman@uq.edu.au*
A/Prof. Jane Black *Monash University* *Melbourne, VIC* *jane.black@monash.edu*	**Prof. Frank Bloomfield** *The University of Auckland* *Auckland, New Zealand* *f.bloomfield@auckland.ac.nz*	**Prof. Alan Bocking** *Mount Sinai Hospital* *Toronto, ON, Canada* *ABocking@mtsinai.on.ca*
Dr. Nadine Brew *Hudson Institute* *Melbourne, VIC* *nadine.brew@hudson.org.au*	**Ms. Emma Buckels** *The University of Auckland* *Auckland, New Zealand* *e.buckels@Auckland.ac.nz*	**Miss. Lara Bush** *Hudson Institute* *Melbourne, VIC* *lebus2@student.monash.edu*
Ms. Yan Yee (Kyra) Chan *Hudson Institute* *Melbourne, VIC* *yycha32@student.monash.edu*	**Dr. Kirat Chand** *The University of Queensland* *Brisbane, QLD* *k.chand@uq.edu.au*	**Dr. Emily Cohen** *Hudson Institute* *Melbourne, VIC* *emily.cohen@monash.edu*
Prof. Tim Cole *Monash University* *Melbourne, VIC* *tim.cole@monash.edu*	**Dr. Kelly Crossley** *Hudson Institute* *Melbourne, VIC* *kelly.crossley@monash.edu*	**Ms. Angela Cumberland** *University of Newcastle* *Newcastle, NSW* *angela.cumberland@uon.edu.au*
Mr. Jack Darby *The University of Adelaide* *Adelaide, SA* *jack.darby@mymail.unisa.edu.au*	**Dr. Marcus Davey** *The Children's Hospital of Philadelphia* *Philadelphia, PA* *daveym@email.chop.edu*	**Dr. Robert De Matteo** *Monash University* *Melbourne, VIC* *robert.dematteo@monash.edu*
Dr. Hayley Dickinson *Hudson Institute* *Melbourne, VIC* *hayley.dickinson@monash.edu*	**Dr. Yvonne Eiby** *The University of Queensland* *Brisbane, QLD* *y.eiby@uq.edu.au*	**Prof. Dawn Elder** *University of Otago* *Wellington, NZ* *dawn.elder@otago.ac.nz*

Dr. Stacey Ellery *Hudson Institute* *Melbourne, VIC* *stacey.ellery@monash.edu*	**Prof. Abby Fowden** *University of Cambridge* *Cambridge, UK* *alf1000@cam.ac.uk*	**Dr. Robert Galinsky** *The University of Auckland* *Auckland, New Zealand* *r.galinsky@auckland.ac.nz*
Dr. Linda Gallo *The University of Queensland* *Brisbane, QLD* *linda.gallo@mater.uq.edu.au*	**Dr. Kathy Gatford** *The University of Adelaide* *Adelaide, SA* *kathy.gatford@adelaide.edu.au*	**Ms. Kate Goasdoue** *The University of Queensland* *Brisbane, QLD* *kate.goasdoue@uqconnect.edu.au*
Prof. Alistair Gunn *The University of Auckland* *Auckland, New Zealand* *aj.gunn@auckland.ac.nz*	**Prof. Richard Harding** *Monash University* *Melbourne, VIC* *richard.harding@monash.edu*	**Prof. Jonathan Hirst** *University of Newcastle* *Newcastle, NSW* *jon.hirst@newcastle.edu.au*
Dr. Nicolette Hodyl *The University of Adelaide* *Adelaide, SA* *nicolette.hodyl@adelaide.edu.au*	**Prof. Stuart Hooper** *Hudson Institute* *Melbourne, VIC* *stuart.hooper@monash.edu*	**Prof. Rosemary Horne** *Hudson Institute* *Melbourne, VIC* *rosemary.horne@monash.edu*
Mr. Mikee Inocencio *Hudson Institute* *Melbourne, VIC* *ishmael.inocencio@monash.au*	**Dr. Anne Jaquiery** *The University of Auckland* *Auckland, New Zealand* *a.jaquiery@auckland.ac.nz*	**Dr. Yogavijayan Kandasamy** *James Cook University* *Townsville, QLD* *Yoga.Kandasamy@health.qld.gov.au*
Dr. Marc Kelly *SWAHD* *Sydney, NSW* *marckellyinoz@gmail.com*	**Dr Kelly Kenna** *Murdoch Children's Research Institute* *Melbourne, VIC* *Kelly.kenna@mcri.edu.au*	**Prof. Alison Kent** *Australian National University* *Canberra, ACT* *Alison.kent@act.gov.au*
Dr. Lauren Kerr *Hudson Institute* *Melbourne, VIC* *lauren.kerr@monash.edu*	**Mr. Ruhan Kruger** *Hudson Institute* *Melbourne, VIC* *rfkru1@student.monash.edu*	**Dr. Domenic LaRosa** *Hudson Institute* *Melbourne, VIC* *domenic.larosa@monash.edu*
Dr. Leo Leader *The University of NSW* *Sydney, NSW* *l.leader@unsw.edu.au*	**Dr. Barbara Lingwood** *The University of Queensland* *Brisbane, QLD* *b.lingwood@uq.edu.au*	**Dr. Alessandra Lio** *Hudson Institute* *Melbourne, VIC*
Mr. Mitchell Lock *The University of South Australia* *Adelaide, SA* *Mitchell.lock@mymail.unisa.edu.au*	**Dr. Samantha Louey** *Oregon Health and Science University* *Portland, OR, USA* *loueys@ohsu.edu*	**Mrs. Tanzila Mahzabin** *The University of Western Australia* *Perth, WA* *tanzila.mahzabin@uwa.edu.au*
Prof. Gert Maritz *University of Western Cape* *Bellville, South Africa* *gmaritz@uwc.ac.za*	**Dr. Courtney McDonald** *Hudson Institute* *Melbourne, VIC* *courtney.mcdonald@hudson.org.au*	**Miss. Kirsten McInerney** *The University of Newcastle* *Newcastle, NSW* *kirsten.mcinerney@uon.edu.au*

Ms. Stephanie Miller *The University of Queensland* *Brisbane, QLD* *s.odriscoll@uq.edu.au*	**A/Prof. Hawley Montgomery-Downs** *West Virginia University* *Morgantown, West Virginia* *Hawley.Montgomery-Downs@mail.wvu.edu*	**A/Prof. Karen Moritz** *The University of Queensland* *Brisbane, QLD* *k.moritz@uq.edu.au*
Prof. Janna Morrison *The University of South Australia* *Adelaide, SA* *janna.morrison@unisa.edu.au*	**A/Prof. Tim Moss** *Hudson Institute* *Melbourne, VIC* *tim.moss@monash.edu*	**Ms. Alison Moxham** *Hudson Institute* *Melbourne, VIC* *alison.moxham@monash.edu*
Prof. Peter Nathanielsz *University of Wyoming* *Wyoming, USA* *Peter.Nathanielsz@uwyo.edu*	**Dr. Ilias Nitsos** *Hudson Institute* *Melbourne, VIC* *ilias.nitsos@hudson.org.au*	**Dr. Stephen O'Callaghan** *Darwin Private Hospital* *Tiwi, NT* *stephen@nfm.com.au*
Prof. Julie Owens *The University of Adelaide* *Adelaide, SA* *julie.owens@adelaide.edu.au*	**Ms. Paris Papagianis** *Hudson Institute* *Melbourne, VIC* *paris.papagianis@monash.edu*	**Prof. Helena Parkington** *Monash University* *Melbourne, VIC* *helena.parkington@monash.edu*
Dr. Atul Patel *The Nairobi Hospital* *Nairobi, Kenya* *dratulau@hotmail.com*	**Miss. Madison Paton** *Hudson Institute* *Melbourne, VIC* *madison.paton@monash.edu*	**Prof. Jane Pillow** *The University of Western Australia* *Perth, WA* *jane.pillow@uwa.edu.au*
Dr. Gavin Pinniger *The University of Western Australia* *Perth, WA* *gavin.pinniger@uwa.edu.au*	**Dr. Julia Pitcher** *The University of Adelaide* *Adelaide, SA* *julia.pitcher@adelaide.edu.au*	**Dr. Graeme Polglase** *Hudson Institute* *Melbourne, VIC* *graeme.polglase@monash.edu*
Prof. Sandra Rees *University of Melbourne* *Melbourne, VIC* *s.rees@unimelb.edu.au*	**Dr. Anna Rocha** *Hudson Institute* *Melbourne, VIC* *anna.rocha@monash.edu*	**Dr. Donna Rudd** *James Cook University* *Townsville, QLD* *donna.rudd@jcu.edu.au*
Prof. Dan Rurak *University of British Columbia* *Vancouver, BC, Canada* *drurak@cw.bc.ca*	**Miss. Riana Samuel** *Hudson Institute* *Melbourne, VIC* *rsam16@student.monash.edu*	**Ms. Julia Shaw** *The University of Newcastle* *Newcastle, NSW* *julia.shaw@uon.edu.au*
Dr. Vijay Shingde *University of Sydney* *Sydney, NSW* *vijayshingde@Hotmail.com*	**A/Prof. Gurmmet Singh** *Menzies School of Health Research* *Casaurina, NT* *gurmeet.singh@Menzies.edu.au*	**Prof. Roger Smith** *University of Newcastle* *Newcastle, NSW* *Roger.Smith@newcastle.edu.au*
A/Prof. John Smoleniec *Liverpool Hospital* *Sydney, NSW* *john.smoleniec@sswahs.nsw.gov.au*	**A/Prof. Joe Smolich** *Murdoch Children's Research Institute* *Melbourne, VIC* *joe.smolich@mcri.edu.au*	**Ms. Jia Yin Soo** *The University of South Australia* *Adelaide, SA* *soojy002@mymail.unisa.edu.au*

Dr. Foula Sozo *Monash University* *Melbourne, VIC* *foula.sozo@monash.edu*	**A/Prof. Michael Stark** *The University of Adelaide* *Adelaide, SA* *michael.stark@adelaide.edu.au*	**Ms. Fiona Stenning** *Hudson Institute* *Melbourne, VIC* *fiona.stenning@monash.au*
Prof. Keiji Suzuki *Tokai University* *Kanagawa, Japan* *dkswnd@tokai-u.jp*	**Ms. Kai Yie Tay** *The University of Auckland* *Auckland, New Zealand* *k.tay@auckland.ac.nz*	**A/Prof. Christiane Theda** *Royal Women's Hospital* *Melbourne, VIC* *christiane.theda@thewomens.org.au*
A/Prof. David Todd *Centenary Hospital* *Canberra, ACT* *david.todd@act.gov.au*	**Dr. Mary Tolcos** *RMIT University* *Melbourne, VIC* *mary.tolcos@rmit.edu.au*	**Ms. Amanda Vrselja** *Monash University* *Melbourne, VIC* *amanda.vrselja@monash.edu*
Prof. David Walker *Hudson Institute* *Melbourne, VIC* *david.walker@monash.edu*	**Dr. Megan Wallace** *Hudson Institute* *Melbourne, VIC* *megan.wallace@monash.edu*	**Ms. Sarah Walton** *The University of Queensland* *Brisbane, QLD* *sarah.walton@uqconnect.edu.au*
Prof. Mary Wlodek *The University of Melbourne* *Melbourne, VIC* *m.wlodek@unimelb.edu.au*	**Ms. Amy Woolridge** *University of Adelaide* *Adelaide, SA* *amy.wooldridge@student.adelaide.edu.au*	**Prof. Ian Wright** *The University of Newcastle* *Newcastle, NSW* *ian.wright@newcastle.edu.au*
Dr. Stephanie Yiallourou *Hudson Institute* *Melbourne, VIC* *stephanie.yiallourou@monash.edu*	**Ms. Valerie Zahra** *Hudson Institute* *Melbourne, VIC* *valerie.zahra@monash.edu*	**Prof. Tamás Zakár** *Hunter Medical Research Institute* *Newcastle, NSW* *tamas.Zakar@newcastle.edu.au*