29th Annual Meeting of The Fetal and Neonatal Workshop of Australia and New Zealand

Monash University Law Chambers
555 Lonsdale St, Melbourne, Victoria
16-17 April, 2015

2015 Organising Committee

Rob De Matteo	Monash University
Mary Tolcos	The Ritchie Centre
Richard Harding	Monash University

The FNWANZ is affiliated with the Perinatal Society of Australia and New Zealand Ltd

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FNWANZ Programme Outline

Monash University Law Chambers
555 Lonsdale Street
Melbourne, Victoria

THURSDAY 16TH APRIL
8.30am-9.30am **.....Registration**
9.30am-11.16am **.....Session 1**
11.16am-11.45am **.....Morning Tea**
11.45am-1.30pm **.....Session 2**
1.30pm-2.30pm **.....Lunch**
2.30pm-4.01pm **.....Session 3**
4.01pm-4.30pm **.....Afternoon Tea**
4.30pm-6.01pm **.....Session 4**
7.15pm **.....Conference Dinner** **Lady Cutler Show Boat** **Central Pier, Harbour Esplanade, Docklands**

FRIDAY 17TH APRIL
8.30am-9.15am **..... Registration**
9.15am-10.55am **..... Session 5**
10.550am-11.25pm **..... Morning Tea**
11.25am-1.05pm **..... Session 6**
1.05pm-2.00pm **..... Lunch**
2.00pm-3.30pm **..... Session 7**
3.30pm-4.00pm **..... Afternoon Tea**
4.00pm-5.35pm **..... Session 8**
5.35pm **..... Presentation of prizes**
5.40pm **..... Workshop 2016**
5.45pm **..... Close of Workshop 2015**

FNWANZ Scientific Programme - 2015

DAY 1 - THURSDAY 16th APRIL

Registration: 8.30am-9.30am

**= Short orals; E=Early PhD, L=Late PhD, ECR= Early Career Researcher*

Session 1: Chairs – Mary Tolcos and Michael Stark			
9:30	**A1**	Laura Bennet	The effect of magnesium sulphate on post-asphyxial preterm fetal seizures
9:45	**A2**	Robert Galinsky (ECR)	$MgSO_4$ is not neuroprotective against asphyxia-induced brain injury in preterm fetal sheep
10:00	**A3**	Christopher Lear (E)	Dexamethasone induced hyperglycaemia during asphyxia is associated with severe cystic white and grey matter brain injury in preterm fetal sheep
10:15	**A4**	Jingang Li (L)	Early administration of cord blood cell therapy to reduce preterm brain injury
10:30	**A5**	Samantha Barton (L)	The effect of intrauterine inflammation on the ventilated preterm lamb brain when administered prior to betamethasone
10:45	***A6**	Nadia Hale	Cortical growth in fetal sheep during gyrification
10:53	***A7**	Madison Paton (E)	Umbilical cord blood stem cells: a new line of defence against cerebral palsy
11:01	**General discussion**		

Morning tea: 11.16am-11.45am

Session 2: Chairs – Karen Moritz and Frank Bloomfield			
11:45	**A8**	Jessica Briffa (E)	Normal lactational environment in rats enhances offspring growth and plasma leptin concentrations, but does not alter renal leptin signaling mediator expression after uteroplacental insufficiency
12:00	**A9**	Sarah Delforce (E)	Activation of the renin angiotensin system during decidualisation
12:15	**A10**	Kirsty Pringle	Preterm birth and the decidual/amnion renin-angiotensin system
12:30	**A11**	Sarah Walton (L)	Late gestational hypoxia alters renal structure and function in male but not female mouse offspring
12:45	**A12**	Dana Ryan (L)	Does preterm birth lead to reduced podocyte number in the kidney?
1:00	**A13**	Julia Shaw (E)	Preterm male guinea pig juveniles exhibit hyperactive behaviour
1:15	**General discussion**		

Lunch: 1.30pm-2.30pm

Session 3: Chairs – David Todd and Beverly Muhlhausler			
2.30	**A14**	Kyungjoon Lim (ECR)	Trans-generational effect of maternal obesity on the programming of hypertension: altered leptin signalling pathway in the central nervous system
2.45	**A15**	Erandi Hewawasam (E)	miRNA biomarkers for early prediction of adverse pregnancy outcomes in obese pregnant women
3.00	**A16**	Sabiha Chowdhury (E)	Impact of maternal obesity in rat offspring kidney
3.15	**A17**	Jack Darby (E)	The heart is resistant to increased glucose availability in late gestation
3.30	***A18**	Dayana Mahizir (E)	High fat diet exacerbates glucose intolerance in pregnant females born small
3:38	***A19**	Jessica Gugusheff (ECR)	A maternal 'junk food' diet alters mu-opioid receptor mRNA expression in late but not early postnatal development in female offspring
3:46	**General discussion**		

Afternoon tea: 4.01pm-4.30pm

Session 4: Chairs – Nicolette Hodyl and Jeff Craig			
4.30	**A20**	Jacinta Kalisch-Smith (E)	Peri-conceptional alcohol exposure does not alter pre-implantation phenotypes but can affect trophoblast differentiation in culture
4.45	**A21**	Diana Zanfirache (E)	Behavioural correlates of periconceptional ethanol exposure in aged offspring
5.00	**A22**	Lisa Yamada (L)	Nicotine exposure in early pregnancy: mid-gestational growth restriction correlates with differential mRNA and microRNA expression
5:15	**A23**	Richard Schlegel (L)	The effect of maternal hypomagnesaemia on fetal development and the programming of adult disease
5:30	***A24**	Lisa Akison (ECR)	Prenatal alcohol exposure and female reproductive health – potential impacts on the fertile life span of offspring
5:38	***A25**	Jeffrey Craig	Do low, moderate and high patterns of prenatal alcohol consumption influence placental epigenetics?
5.46	**General discussion**		

Conference dinner: Lady Cutler Show Boat, 7.15pm

DAY 2 - FRIDAY 17th APRIL

Registration: 8.30-9.15am

**= Short orals; E=Early PhD, L=Late PhD, ECR= Early Career Researcher, M=Masters*

Session 5: Chairs – Ian Wright and Yvonne Eiby			
9:15	**A26**	Yvonne Eiby	Low cardiac preload is detrimental to cardiovascular function and cerebral blood flow in preterm piglets
9:30	**A27**	Joe Smolich	Discordance between phasic changes in the aorto-pulmonary pressure difference and ductal blood flow during the preterm birth transition
9:45	**A28**	Rebecca Dyson (ECR)	Vascular dysfunction in the newborn: contribution of the gasotransmitters (and their interactions)
10:00	**A29**	Stacey Vranas (E)	The effects of intrauterine growth restriction on adult heart structure in an ovine model
10:15	**A30**	Paul Lombardo (L)	An evaluation of the effects of moderate preterm birth on the heart and major arteries of male lambs from birth to twelve months of age using serial ultrasound imaging
10:30	***A31**	Corinna Binder-Heschl	Effect of prenatal caffeine infusion on the newborn cardiovascular system at birth
10:38	**General discussion**		

Morning tea: 10:55am-11.25am

Session 6: Chairs – Jon Hirst and Foula Sozo			
11:25	**A32**	Jia Yin Soo (E)	Inflammatory and profibrotic markers in the lung of growth restricted offspring before and after birth
11:40	**A33**	Vicki Clifton	Identification of seven different isoforms of the glucocorticoid receptor in Guinea Pig placenta: relationship to preterm delivery, sex and betamethasone exposure
11:55	**A34**	Sandra Orgeig	Increased prolyl hydroxylase and decreased glucocorticoid receptor are related to decreased surfactant protein in the lung of the chronically hypoxemic growth restricted sheep fetus
12:10	**A35**	Bennet Seow (L)	Comparative effects of endogenous and synthetic glucocorticoid steroids during mammalian lung development
12:25	**A36**	Kirsten McInerney (E)	Adrenal contribution to neurosteroidogenesis in the neonate: effect of preterm birth and progesterone replacement
12:40	***A37**	Tamás Zakár	DNA methylation controls corticotropin releasing hormone (CRH) expression in human trophoblast cells
12:48	**General discussion**		

Lunch: 1.05pm-2.00pm

Session 7: Chairs – Jane Pillow and Keiji Suzuki			
2:00	**A38**	Erin McGillick (L)	Maternal antioxidant treatment increases expression of genes regulating hypoxia signalling, sodium movement & surfactant maturation in the fetal lung
2:15	**A39**	Ishmael Inocencio (E)	Ultra-high dose nanoparticles increased markers of lung injury and inflammation in the preterm lamb
2:30	**A40**	Paris Papagianis (E)	Optimising continuous positive airway pressure for preterm infants
2:45	**A41**	Lauren Kerr (ECR)	Sustained inflations impact lung aeration at birth
3:00	**A42**	Domenic LaRosa (ECR)	Determining the optimal dose of EPO to reduce ventilator induced lung injury
3:15	**General discussion**		

Afternoon tea: 3.30pm-4.00pm

Session 8: Chairs – Laura Bennet and Tamás Zakár			
4:00	**A43**	Emily Cohen (E)	Size at birth and gender influence cerebral oxygenation in preterm neonates during the first three days of life
4:15	**A44**	Kathryn Martinello (M)	Gestational age related alterations in interleukin-6 *trans*-signalling may predispose to bronchopulmonary dysplasia
4:30	**A45**	Nadia Bellofiore (E)	Pseudopregnancy induction in the spiny mouse (*Acomys cahirinus*)
4:45	***A46**	Tamara Yawno	Progesterone treatment of the growth restricted fetus - a natural therapy
4:53	***A47**	Sogand Hosseini (M)	Maternal stress in pregnancy reduced mRNA expression of placental growth related factors
5:01	***A48**	Danielle Burgess (E)	Does periconceptional ethanol exposure in the rat offspring alter the hypothalamic-pituitary-adrenal axis and subsequent stress responses?
5:09	***A49**	Courtney McDonald (ECR)	How does neuroinflammation drive brain injury following hypoxia-ischemia?
5:17	**General discussion**		
5:35	**Presentation of Prizes**		
5:40	**Fetal and Neonatal Workshop 2016**		
5:45	**Close of Workshop**		

Information for delegates

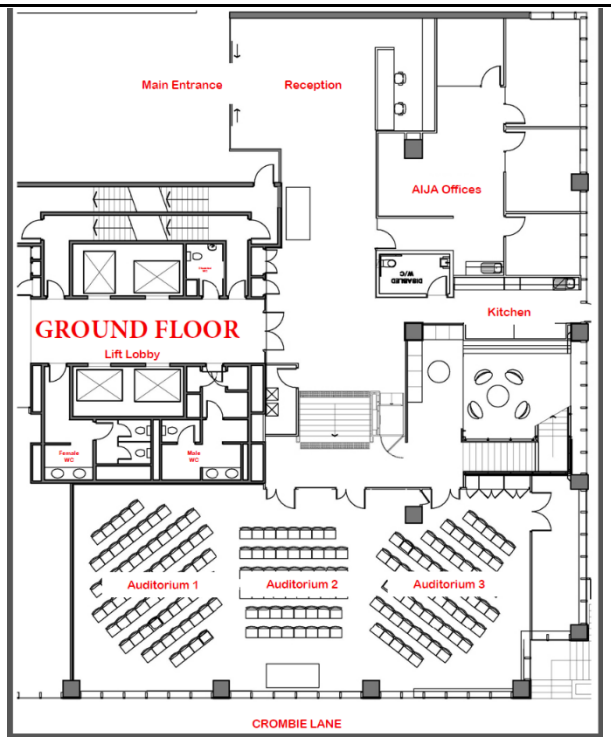
Monash University Law chambers

555 Lonsdale Street (on the corner of Lonsdale Street and Crombie Lane)
Melbourne VIC 3000

Train Stations:
Southern Cross - 6 minute walk
Flagstaff - 3 minute walk

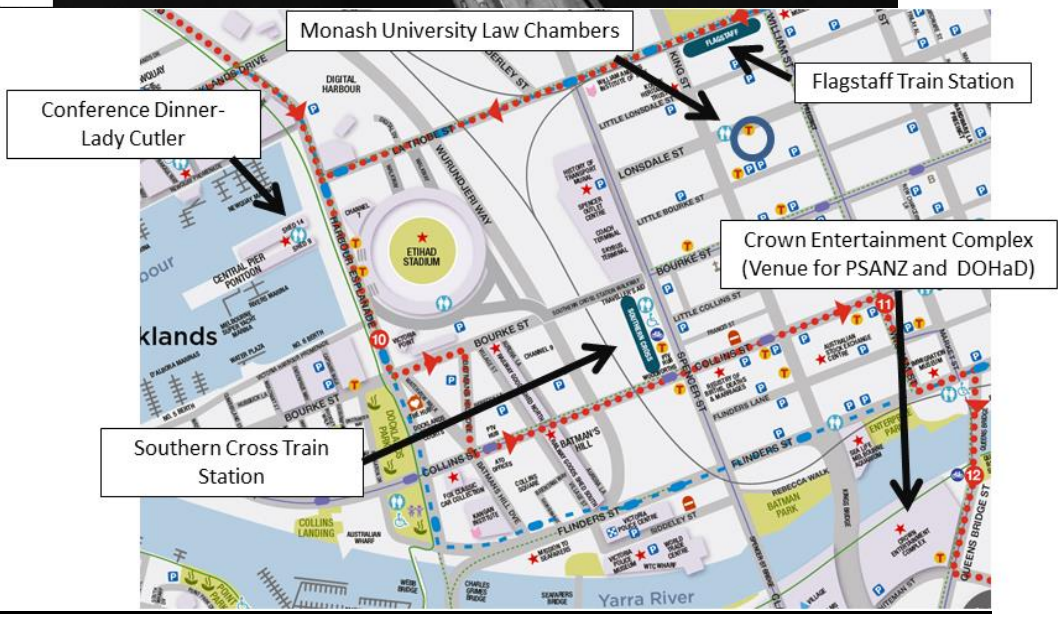
Wireless Access:

1. Via eduroam (eduroam allows students and staff at participating institutions to gain secure access to wireless networking)
2. Use the following information
   Username (Authcate): pvweb1
   Password: mulc2015



Location of Lady Cutler Show Boat

Central Pier, Harbour Esplanade, Docklands



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;

Perinatal Society of Australia & New Zealand

Medicine, Nursing and Health Sciences

Anatomy and Developmental Biology

School of Biomedical Sciences, Monash University, Clayton campus

DANONE NUTRICIA

Early Life Nutrition

Session 1

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11:01	**General discussion**		

The effect of magnesium sulphate on post-asphyxial preterm fetal seizures

Vittoria Draghi, Robert Galinsky, Chris Lear, Alistair Jan Gunn, Laura Bennet

[1]Fetal Physiology and Neuroscience group, Department of Physiology, The University of Auckland, Auckland, New Zealand.
l.bennet@auckland.ac.nz

Background: Magnesium sulphate ($MgSO_4$), given to pregnant mothers to delay preterm labour, may provide perinatal neuroprotection. Seizures after asphyxia are common, and may contribute to brain injury. $MgSO_4$ can prevent maternal seizures in pre-eclampsia. We do not know the effect of $MgSO_4$ on perinatal seizures.

Aims/Hypothesis: To examine the hypothesis that $MgSO_4$ would prevent or significantly reduce post-asphyxial seizures in preterm fetal sheep.

Methods: Fetal sheep at 103 days gestation received either $MgSO_4$ (n=7) as a 160 mg bolus, followed by 48 mg/h constant infusion, or equivalent volume of saline (n=8). Treatment started 24 hours before asphyxia induced by 25 min of complete umbilical cord occlusion (UCO), and was continued until 24 hours post-UCO. Fetuses were studied for 3 days post-UCO. EEG was recorded continuously at 1024Hz for seizure analysis. Seizures were visually identified as high amplitude sudden, repetitive, evolving wave forms. Total seizure duration, numbers of seizures, minutes spent seizing per hour, peak amplitude and individual durations were assessed.

Results: $MgSO_4$ did not stop seizures, or their start time (14.4 ± 4.1 vs. 13.5±3.3 hours, $MgSO_4$ vs control), overall amplitude (95.0±17.4 144.0±18.0 μV) or overall individual duration (69.0±7.2 vs.74.0±6.0 sec). $MgSO_4$ did shorten total duration of seizures (25.0±7.0 vs. 28.2±6.2 hours, $P<0.05$) and reduced total numbers of seizures (33.3± 6.4 vs. 65.0±20.0, $P<0.05$). However, seizure activity differed over time. Hourly analysis (post-UCO) showed that the amplitude of seizures was lower between 33-42 hours ($P<0.05$), individual seizures were shorter between 36-40 hours ($P<0.05$), there were fewer seizures per hour between18-37 hours ($P<0.05$), and time spent hour making seizures per hour was lower between 17-34 hours ($P<0.05$). Overall analysis suggested a diurnal rhythm in seizure activity.

Conclusions: $MgSO_4$ is a glutamate antagonist and can reduce the permeability of mitochondrial pores, which open when oxidative metabolism is compromised leading cellular dysfunction. $MgSO_4$ at the dose given was insufficient to prevent the initial abrupt loss of oxidative metabolism and associated seizures. However, there was an effect on seizures starting ~12 hours later when brain levels of glutamate and GABA are known to fall in a circadian manner. Seizures increased after $MgSO_4$ infusion stopped, but they were smaller and finished earlier. Our data show that $MgSO_4$ can ameliorate the post-asphyxial seizure burden, that there may be a circadian component to seizure activity, and that sustained infusion of $MgSO_4$ throughout the secondary phase of loss of oxidative metabolism may further reduce seizure activity.

$MgSO_4$ is not neuroprotective against asphyxia-induced brain injury in preterm fetal sheep

Joanna Tse, Joanne O Davidson, Christopher A Lear, Paul P Drury, Guido Wassink, Lotte Van den Heuij, Laura Bennet, Alistair Jan Gunn, Robert Galinsky

The Department of Physiology, The University of Auckland, Auckland, New Zealand.
r.galinsky@auckland.ac.nz

Background: There is an important, unmet need to improve the outcome of neonatal encephalopathy in preterm infants. It remains controversial whether $MgSO_4$ is clinically neuroprotective, and thus, it is unclear whether it would be appropriate to use $MgSO_4$ for treatment / prevention of encephalopathy in preterm infants.

Aim: To test the neuroprotective efficacy of $MgSO_4$ for hypoxic-ischemic encephalopathy after profound asphyxia in preterm fetal sheep.

Methods: At 104 ± 1 days (0.7) of gestation, fetal sheep were randomly assigned to receive a continuous infusion of i.v. $MgSO_4$ (n=7) or vehicle (control; n=10) starting 24 hours before 25 minutes of complete umbilical cord occlusion, and continued for 24 hours after occlusion. Mean arterial pressure (MAP), fetal heart rate (FHR), and carotid blood flow (CaBF), fetal electroencephalography (EEG) and movement (nuchal electromyography (EMG)) were measured continuously. 72 h after occlusion, fetal brains were processed for neuropathological assessment of the subcortical grey matter, including the mid-striatum, hippocampus, dentate gyrus and thalamus, and intragyral and periventricular white matter.

Results: $MgSO_4$ treatment increased plasma magnesium levels from 0.78 to 1.89 mmol/L ($p<0.05$). The $MgSO_4$ infusion before asphyxia was associated with reduced FHR and EEG power ($p<0.05$; $MgSO_4$ vs. control). $MgSO_4$ did not affect neuronal survival in subcortical grey matter, numbers of activated microglia or numbers of reactive astrocytes. $MgSO_4$ treated fetuses had reduced numbers of oligodendrocytes in the intragyral white matter ($p<0.05$).

Conclusions: A clinically comparable dose of $MgSO_4$ was associated with reduced FHR and EEG power before asphyxia. $MgSO_4$ did not reduce asphyxia-induced preterm brain injury and may increase oligodendrocyte loss.

Dexamethasone induced hyperglycaemia during asphyxia is associated with severe cystic white and grey matter brain injury in preterm fetal sheep

Christopher A. Lear, Joanne O. Davidson, Robert Galinsky, Alistair J. Gunn, Laura Bennet

Fetal Physiology and Neuroscience Group, The University of Auckland, New Zealand.
christopher.lear@auckland.ac.nz

Background: Antenatal glucocorticoids are given routinely to mothers at risk of preterm labour. A side effect of this treatment is hyperglycaemia. In human pregnancy hyperglycaemia is associated with adverse neonatal outcomes; however, it is unclear whether this link is causal. Conversely, in newborn rodents, both synthetic glucocorticoids and hyperglycaemia before hypoxia-ischaemia are highly neuroprotective, but there is limited evidence from large animal studies.

Aims/Hypothesis: To test the hypothesis that maternal dexamethasone (DEX) modulates asphyxial brain injury through the induction of hyperglycaemia.

Methods: At 0.7 of gestation, chronically instrumented singleton sheep fetuses were exposed to maternal injection of 12 mg DEX i.m. (n=7) or maternal saline (n=7), or maternal saline plus fetal i.v. infusion of glucose dissolved in saline (2 mmol/ml, Glucose group, n=6), titrated to increase fetal plasma glucose levels to those observed in the DEX group. Fetal asphyxia was induced 4 hours later by 25 minutes of complete umbilical cord occlusion. Fetal physiological parameters were continuously monitored until 7 days after asphyxia. At post-mortem, fetal brains were taken for histological assessment.

Results: DEX and glucose treatment increased fetal blood glucose levels before asphyxia (DEX: 2.0±0.2 and Glucose: 2.4±0.3 *vs.* saline: 1.0±0.1 mmol/l, $p<0.05$). DEX or glucose treatment did not affect fetal arterial blood pressure or metabolic acidosis during occlusion. Occlusion with saline was associated with subcortical neuronal loss ($p<0.05$) and diffuse white matter injury, including loss of mature oligodendrocytes ($p<0.05$) without cystic lesions. DEX and Glucose were associated with increased grey and white matter injury compared to saline treatment, including severe cystic infarcts in the thalamus and periventricular white matter in all DEX and Glucose fetuses ($p<0.001$, Fisher exact test). Within the DEX and Glucose groups higher peak plasma glucose values were correlated with more extensive injury, including cystic damage of the striatum and sagittal gyrus. Greater injury was in turn associated with greater seizure burden ($p<0.05$) and greater delayed increase in cortical impedance ($p<0.05$) during the 7 day recovery period.

Conclusions: Maternal DEX injection and fetal hyperglycaemia in a profile that is similar to that induced by DEX were unexpectedly associated with dramatic, dose-related exacerbation of neural injury after subsequent fetal asphyxia, including severe cystic infarction. These data strongly suggest that the clinical association of perinatal hyperglycaemia with adverse outcomes is causal.

Early administration of cord blood cell therapy to reduce preterm brain injury

Jingang Li, Tamara Yawno , Amy Sutherland, Jan Loose, Ilias Nitsos, Flora Wong, Graham Jenkin, Suzanne L Miller

The Ritchie Centre, MIMR-PHI Institute, Monash University, VIC 3168, Australia.
jingang.li@monash.edu

Background: Preterm infants, particularly infants born under 32 weeks of gestational age, are at high risk of developing brain damage. There is increasing evidence that umbilical cord blood (UCB) may have therapeutic potential in improving brain injury in rodent model of neonatal asphyxia. However, few studies have investigated the efficacy and mechanisms of action of UCB in the preterm brain. This study aimed to examine the effectiveness in protecting preterm brain of allogeneic UCBC administration to preterm fetal sheep following Umbilical Cord Occlusion (UCO).

Methods: UCO or sham occlusion was performed for 25 minutes to fetal sheep at 98-103 d gestational age (~0.65 gestation). 50 million CFSE-labeled UCBCs, derived from term or preterm ovine cord blood, or saline were administered intravenously to the fetus at 12 h or 5 d after UCO. Ten days following UCO, animals were euthanased for brain collection and analysis.

Results: Brain histology revealed that CFSE-tagged cells given i.v. were detected in the preterm brain. UCO reduced Olig-2 positive oligodendrocytes in the white matter, while both term and preterm derived UCBC administration at 12 h prevented the reduction. Additionally, UCO animals showed a significant increase in Iba-1 positive microglia in the white matter and UCBCs significantly ameliorated these effects. UCBCs at 5 d failed to show these, potentially, therapeutic effects.

Conclusion: Allogeneic UCBC administration at 12 h, but not at 5 days, after ischemia, reduces preterm brain injury.

The effect of intrauterine inflammation on the ventilated preterm lamb brain when administered prior to betamethasone

Samantha Barton[1], Mary Tolcos[1], Tim Moss[1,2], Stuart Hooper[1,2], Martin Kluckow[3], Andy Gill[4] & Graeme Polglase[1,2]

[1]The Ritchie Centre, MIMR-PHI Institute of Medical Research, Clayton VIC 3168; [2]Department of Obstetrics & Gynecology, Monash University, Clayton VIC 3168; [3]Department of Neonatology, Royal North Shore Hospital, Sydney, Australia; [4]Centre for Neonatal Research and Education, University of Western Australia, Perth, Australia.
samantha.barton@monash.edu

Background: Chorioamnionitis is common in preterm infants and increases the risk of white matter (WM) abnormalities including cerebral palsy. Ventilation further increases WM injury, however the interaction with betamethasone (BM) is unknown.

Aims/Hypothesis: We aimed to investigate the interaction of chorioamnionitis, BM and ventilation on cerebral haemodynamics and WM injury in preterm lambs.

Methods: Pregnant ewes (0.85 gestation) received BM (IM; 11.4mg) 48 and 24 h prior to delivery/ventilation. Two groups received intra-amniotic lipopolysaccharide (LPS; 10mg) 3 h prior to BM. 4 groups were analysed: 1. BM Unventilated control (UVC_{BM}; n=6); 2. BM+Ventilation ($Vent_{BM}$; n=7); 3. LPSUVC_{BM} (n=6); 4. LPS$Vent_{BM}$ (n=7). Lambs were instrumented for measurement of cerebral haemodynamics prior to receiving injurious ventilation for 15 min and then a gentle strategy for a further 75 min (A 2-way RM ANOVA was used to statistical analysis). At autopsy the brains were collected and inflammation and vascular leakage was assessed within the periventricular and subcortical WM using qRT-PCR and immunohistochemistry (A 2-way ANOVA was used for statistical analysis).

Results: Ventilatory and blood-gas variables were not different between groups throughout the ventilation studies. Mean carotid blood flow (CBF) tended lower in LPS$Vent_{BM}$ lambs compared to $Vent_{BM}$ lambs throughout ventilation, which was caused by negative minimum CBF during diastole, indicative of ductal steal. IL-1b and IL-6 mRNA levels decreased in both Vent groups compared to both UVC groups yet IL-8 mRNA levels increased in both LPS treated groups compared to UVC_{BM} and $Vent_{BM}$ groups ($p<0.05$ for all)**.** There was no difference in area of WM occupied by microglial aggregation nor the density of microglia within aggregations. The number of vessel profiles with protein extravasation was lower after Vent compared to both UVC groups in the periventricular and subcortical WM ($p<0.05$).

Conclusions: The presence of chorioamnionitis prior to BM administration resulted in adverse cerebral haemodynamics during the initial ventilation after preterm birth. Both BM and chorioamnionitis appeared to induce tolerance in the brain to further injury from ventilation evidenced by reduced inflammatory markers and vascular leakage.

Gyrification in fetal sheep during cortical growth: impact of reduced umbilical blood flow

Nadia Hale[1], Meghan Boomghardt[1], Mary Tolcos[1] Joanne M Britto[2], David Walker[1]

[1]The Ritchie Centre, MIMR-PHI Institute of Medical Research, Melbourne, Australia; [2]The Florey Institute of Neuroscience and Mental Health, University of Melbourne, Australia.
Nadia.hale@mimr-phi.org

Background: The processes by which cortical folding occurs during development is relatively unknown. While *in utero* conditions that impact fetal growth have been intensively studied, their effects on cortical development, in particular the development of gyri and sulci, have not been fully investigated.

Aims: (i) To identify the initial stages of cortical folding in the fetal sheep; (ii) to determine the impact of single uterine artery ligation (SUAL) on cortical folding and development.

Methods: Brains were collected from fetal sheep time points between 56 and 113 days of gestation (dg; n=20). In another cohort, SUAL was performed at a time between 55 and 70dg, and brains collected at 90dg (n=5); the non-ligated twin (n=5) served as the control. At post-mortem, brain and body weights were taken, and the brains collected for magnetic resonance (MR) imaging (to assess gyrification) and immunohistochemistry (to assess subcortical projection neurons, oligodendrocytes, microglia and apoptosis).

Results: Qualitative assessment revealed that cortical folding starts after 70dg with primary sulcus formation occurring between 70 and 78dg. SUAL did not change brain or body weights compared to controls, but topographically SUAL brains were less folded compared to controls; MR images and immunohistochemical analysis of neural cell populations in sulci vs gyri are currently being assessed.

Conclusion: Gyrification occurs almost entirely between days 70 and 90dg in the sheep. SUAL appears to reduce gyrification. Analysis of neural cell populations in cortical gyri and sulci will identify cellular changes underlying impaired gyrification.

Umbilical cord blood stem cells: a new line of defence against Cerebral Palsy

Paton, M.[1*], McDonald, C.[1*], Aridas J.[1], Yawno, T.[1], Fahey, M.[1,2], Castillo-Melendez, M.[1], Miller, S.[1] and Jenkin, G.[1,3] *Joint first authors.

[1]The Ritchie Centre, MIMR-PHI Institute, Clayton, Australia; [2]Department of Paediatrics, Monash Medical Centre, Clayton, Australia; [3]Obstetrics and Gynaecology, Monash Medical Centre, Clayton, Australia.
madison.paton@monash.edu

Background: Cerebral Palsy (CP) is the most prevalent cause of chronic disability in children. With limited treatments available, many seek overseas stem cell treatment. However, it remains unknown if such treatments are safe, effective, or the likely mechanism of action. In this study we examined whether adherent umbilical cord blood mononuclear cells (UCB-MNCs), including endothelial progenitor cells (EPCs) and mesenchymal stromal cells (MSCs), could reduce neuronal injury following birth asphyxia; a major risk factor of CP.

Aims/Hypothesis: Adherent UBC-MNCs administered 12 or 24 hours following birth asphyxia will prevent and reduce brain injury.

Methods: UCB-MNCs were isolated from term lamb umbilical cord blood, expanded in culture and characterised. $10x10^6$ UCB-MNCs were administered to term lambs 12 or 24 hours following birth asphyxia. MRS was undertaken at 72 hours, lambs were then euthanised, CSF and brains collected.

Results: MSCs and EPCs were identified in UCB-MNC cultures. Animals that received UCB-MNC treatment showed reduced inflammatory cell activation in the brain and increased CSF IL-10. MRS analysis in lambs administered cells 12 and 24 hours following asphyxia revealed a trend towards an increase in N-acetyl aspartate in the brain (NAA; a marker of neuronal integrity) compared to birth asphyxia alone. The 24 hour cohort also showed a reduction in lactate:NAA ratio (a marker of aerobic metabolism) compared to birth asphyxia alone.

Conclusions: Administration of adherent UCB-MNCs, containing EPCs and MSCs, to lambs following birth asphyxia results in improved neuronal integrity and decreased inflammation in the brain. Whilst more research is required, culture expanded UCB-MNCs may represent a useful treatment to prevent the progression of CP following birth asphyxia.

Session 2

Session 2: Chairs – Karen Moritz and Frank Bloomfield			
11:45	**A8**	Jessica Briffa (E)	Normal lactational environment in rats enhances offspring growth and plasma leptin concentrations, but does not alter renal leptin signaling mediator expression after uteroplacental insufficiency
12:00	**A9**	Sarah Delforce (E)	Activation of the renin angiotensin system during decidualisation
12:15	**A10**	Kirsty Pringle	Preterm birth and the decidual/amnion renin-angiotensin system
12:30	**A11**	Sarah Walton (L)	Late gestational hypoxia alters renal structure and function in male but not female mouse offspring
12:45	**A12**	Dana Ryan (L)	Does preterm birth lead to reduced podocyte number in the kidney?
1:00	**A13**	Julia Shaw (E)	Preterm male guinea pig juveniles exhibit hyperactive behaviour
1:15	**General discussion**		

Normal lactational environment in rats enhances offspring growth and plasma leptin concentrations, but does not alter renal leptin signaling mediator expression after uteroplacental insufficiency

Jessica F Briffa[1], Rachael O'Dowd[1], Andrew J Jefferies[1], Karen M Mortiz[2], Andrew J. McAinch[3], Mary E Wlodek[1], Deanne H Hryciw[1]

[1]Department of Physiology, The University of Melbourne, Parkville, VIC, 3010, Australia; [2]School of Biomedical Sciences, The University of Queensland, St. Lucia, QLD, 4072, Australia; [3]Centre For Chronic Disease Prevention and Management, College of Health and Biomedicine, Victoria University, St Albans, VIC 3021, Australia.
jgriffith@student.unimelb.edu.au

Background: Uteroplacental insufficiency is the leading cause of growth restriction in the Western world. Being born small is associated with increased risk of developing adult cardio-renal diseases. Research has established that leptin plays an important role in organ development. We have previously demonstrated that cross-fostering growth restricted offspring onto control mothers improves postnatal (PN) growth, restores nephron deficits and prevents adult hypertension.

Aim: We investigated the effect cross-fostering has on offspring growth, milk intake, plasma leptin concentrations and renal leptin signaling mediator expression.

Methods: Pregnant female WKY rats underwent either sham surgery or bilateral uterine vessel ligation at embryonic day 18 (E18; term = E22) and delivered naturally. At PN1, the offspring were removed from their mother and placed onto either a different control or restricted mother giving rise to four pup-on-mum groups (*Control-on-Control; Control-on-Restricted, Restricted-on-Control; Restricted-on-Restricted*). One cohort of animals was killed at PN7 (during the postnatal leptin surge) to measure changes in plasma leptin and renal leptin signaling mediators, and another cohort was studied during lactation examining offspring growth and milk intake.

Results: Restricted offspring were smaller than control offspring at PN1. Female Restricted-on-Control offspring had delayed accelerated growth compared to male Restricted-on-Control offspring. Cross-fostering restricted offspring onto control mothers restored plasma leptin to Control-on-Control animals' concentrations, which was independent of changes in maternal milk leptin. These findings are likely due to the increased milk intake in Restricted-on-Control offspring. Interestingly, both Control and Restricted mothers suckling restricted offspring had elevated plasma leptin, indicating offspring influences on maternal leptin concentrations. Interestingly, cross-fostering did not alter leptin signaling mediator expression in the kidney.

Conclusions: Our data indicate that normal lactational environment improves growth and restores plasma leptin in Restricted offspring born small, due to increased nutrient delivery, which may contribute to the beneficial effects on growth. However, cross-fostering did not alter leptin signaling mediator expression in the kidney suggesting enhanced renal leptin signaling is not responsible for the improved nephrogenesis observed in Restricted-on-Control offspring.

Activation of the renin angiotensin system during decidualisation

Sarah J. Delforce[1], Kirsty G. Pringle[1] Yu Wang[1], Phillip Logan[2], Murray Mitchell[3] & Eugenie R. Lumbers[1]

[1]School of Biomedical Sciences and Pharmacy, University of Newcastle, NSW; [2]UCSF Centre for Reproductive Sciences, University of California, CA; [3]UQ Centre For Clinical Research, University of Queensland, QLD.
sarah.delforce@uon.edu.au

Background: Decidualisation is critical for implantation and placentation. We have previously shown that decidualisation of human endometrial stromal cells (HESCs) *in vitro* is associated with increased prorenin (*REN*) mRNA expression. Renin is the rate-limiting enzyme of the renin angiotensin system; expression of its precursor, prorenin, is highest in human decidua compared to all other intrauterine tissues. We propose that prorenin is involved in decidualisation by regulating angiogenesis, cell growth and remodelling through downstream factors, such as promyelocytic leukemia zinc finger (PLZF), which promotes proliferation and inhibits apoptosis, and vascular endothelial growth factor (VEGF), a potent angiogenic factor.

Aims: To determine whether the increase in *REN* expression during decidualisation is accompanied by increases in VEGF and PLZF and whether this is due to the hormones, progesterone and estradiol, or stimulation of cAMP (a known regulator of kidney *REN)*.

Methods: HESCs were cultured for 10 days with 1μM medroxyprogesterone acetate (MPA) plus 10nM estradiol-17β (E_2) with or without 0.5mM dibutyryl cyclic AMP (cAMP), along with control. mRNA levels of prorenin (*REN*) and other RAS components, as well as PLZF (*ZBTB16*) and VEGF (*VEGF*) were measured relative to β-actin using the standard curve method. Levels of prorenin protein in the supernatant were measured by ELISA.

Results: *REN, VEGF* and *ZBTB16* expression and prorenin protein levels were significantly upregulated in HESCs following treatment with the combination of E_2, MPA and cAMP in comparison to both the control and E_2 + MPA treatment without cAMP (all $P<0.01$). The expression of angiotensinogen (*AGT*) was significantly increased by treatment with both E_2, MPA +/- cAMP compared to control.

Conclusions: Overall, these findings suggest that decidualisation is associated with the upregulation of *REN, AGT* and prorenin protein and this is primarily driven by cAMP. It also suggests that the increase in prorenin (mRNA and protein) acting through the (pro)renin receptor may be stimulating the anti-apoptotic PLZF and the pro-angiogenic VEGF to maintain decidualisation.

Preterm birth and the decidual/amnion renin-angiotensin system

Kirsty G. Pringle[1], Yu Wang[1], Tamas Zakar[2], Eugenie R. Lumbers[1].

[1]School of Biomedical Sciences and Pharmacy; [2]School of Medicine and Public Health, University of Newcastle and Hunter Medical Research Institute.
Kirsty.Pringle@newcastle.edu.au

Background: The rates of spontaneous preterm labour and premature rupture of membranes are significantly higher in women with male infants. The cause(s) of this sex difference are unknown but it is suggested that intrauterine tissues regulate fetal growth and survival in a sex-specific manner. We have demonstrated that decidua from women carrying a female baby produce higher levels of prorenin during pregnancy and have an increased ability to secrete prorenin.

Aims/Hypothesis: We postulated that prorenin binding to its (pro)renin receptor ((P)RR) acts in a fetal sex specific manner in human amnion to regulate the expression of promyelocytic zinc finger (PLZF), a negative regulator of (P)RR expression, as well as pathways that might influence integrity of the fetal membranes such as transforming growth factor – β1 (TGF-β1).

Methods: To test this hypothesis we incubated freshly collected male and female human amnion in DMEM/F12 medium with exogenous prorenin (0, 5, 50 ng/mL) for 0.5-24h at 37°C.

Results: Male amnion had lower levels of (P)RR ($P < 0.001$) and TGF-β1 ($P = 0.024$) mRNA than female amnion. There were significant correlations between (P)RR and TGF-β1 mRNA levels in both male ($r = 0.395$, $P = 0.012$) and female ($r = 0.717$, $P < 0.001$) amnion explants. In female amnion, (P)RR and TGF-β1 mRNA levels are both positively correlated with p85α-PI3K expression ($r = 0.691$, $P < 0.001$ and $r = 0.601$, $P < 0.001$, respectively). This pathway causes cellular proliferation and inhibits apoptosis. In male amnion, there is an inverse correlation between (P)RR and p85α-PI3K ($r = -0.367$, $P = 0.022$) and only a weak correlation between TGF-β1 and p85α-PI3K ($r = 0.314$, $P = 0.052$). Together these data suggest that the (P)RR/TGF-β1 pathway is suppressed in male amnion.

Conclusions: Our findings demonstrate that there are strong interactions between prorenin, (P)RR and TGF-β1 and that this system has a greater capacity in female amnion to stimulate pro-fibrotic pathways, thus maintaining the integrity of the fetal membranes. More research is needed however to investigate whether this pathway and other pro-fibrotic molecules (collagen, PAI-1 and fibronectin) play a functional role in the fetal sex specific regulation of membrane integrity and whether this pathway is dysregulated in women with preterm premature rupture of membranes.

Late gestational hypoxia alters renal structure and function in male but not female mouse offspring

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

[1]School of Biomedical Sciences, University of Queensland, QLD 4067, Australia; [2]Department of Physiology, Monash University, VIC 3800, Australia; [3]School of Science, University of Queensland, QLD 4343, Australia; [4]Institute for Molecular Bioscience, University of Queensland, QLD 4067, Australia.
sarah.walton@uqconnect.edu.au

Background: Reduced oxygen supply to the fetus is a common gestational perturbation linked to growth restriction and cardiovascular disease. Little is understood regarding the impact of gestational hypoxia on the developing kidney.

Aims/Hypothesis: This study aimed to examine the impact of late maternal hypoxia on the structure and function of the kidney. We hypothesised that offspring would have reduced nephron endowment and altered renal tubule structure, leading to increased risk of renal and cardiovascular dysfunction in later life.

Methods: Pregnant CD1 mice were housed in a hypoxic chamber (12.0% O_2; N=11, HYP) or control (21% O_2; N=11, CON) environment from embryonic day 14.5 to birth (E19.5). A subset of offspring was culled at postnatal day (P) 21 for estimation of nephron number and renal tubule lengths using unbiased stereology. Renal function under basal or dehydration conditions was assessed in 12-month-old offspring via urine collection in metabolic cages. Sections were examined by an expert pathologist blinded to treatment groups. Blood pressure was assessed via radiotelemetry.

Results: Nephron number was reduced by 24% in male HYP offspring (P=0.0006). Total proximal tubule length was increased in male HYP offspring (P=0.007), reflected by increased cortex-to-medulla ratio (P=0.02) at P21. At 12 months, male HYP offspring exhibited mild albuminuria (P=0.009) and minor defects in their ability to concentrate urine when dehydrated. Kidneys of male HYP offspring showed expansion of the mesangial matrix and thickening of glomerular basement membrane compared to CON. No differences in renal structure or function were observed between female CON and HYP offspring. Both male and female HYP offspring had increased mean arterial pressure ($P_{TREATMENT}$<0.0001).

Conclusions: Moderate hypoxia throughout late gestation impaired renal structure and function in male but not female offspring. We are now investigating the basis of the sexually dimorphic alterations seen in the fetal renal and cardiovascular systems.

Does preterm birth lead to reduced podocyte number in the kidney?

Dana Ryan[1], Lynette Moore[2], Victor Puelles Rodriguez[1], John Bertram[1], Mary Jane Black[1]

[1]Anatomy & Developmental Biology, Monash University and [2]Department of Surgical Pathology, South Australia.
Danica.vojisavljevic@monash.edu

Background: The podocyte is a glomerular epithelial cell that plays a crucial role in glomerular filtration. It is generally considered that podocyte proliferation occurs during *in utero* development and that podocyte proliferation is rare after birth. Podocyte depletion increases the risk of developing proteinuria and glomerulosclerosis. Preterm infants are often born when nephrogenesis is ongoing, and thus podocytes are still forming. To date, the effect of preterm birth on podocyte growth is unknown.

Aims/Hypothesis: The specific aims were to examine the effect of preterm birth in the developing human kidney on: the number of podocytes per glomerulus in the kidneys, as well as, to compare the proportion of the different cell types within the glomerulus in term and preterm kidneys.

Methods: Autopsied kidneys embedded in paraffin were serially sectioned (25 serial sections) and 6 glomeruli in the inner cortex region were sampled for podocyte counts. The glomerular cell types were identified using triple immunostaining, with Wilms' tumour 1 antigen (WT1) used to identify podocytes, Von Willebrand factor (vWF) used to identify endothelial cells and DAPI used to stain all nuclei. In order to estimate podocyte number and the number of other cell types, a confocal optical disector approach was used. The glomerular volume was determined using the Cavalieri principle.

To date: These studies are currently in progress. Findings from the glomeruli of an extremely preterm infant (gestational age of 27 weeks at birth and who lived for 34 days after birth) will be compared to infants born at 27 weeks gestation, 31.1 weeks gestation and at term.

Conclusions: We expect that podocyte growth will be impaired as a result of preterm birth, leading to fewer podocytes per glomerulus in the preterm kidney.

Preterm male guinea pig juveniles exhibit hyperactive behaviour

Julia Shaw[1], Hannah Palliser[1], Max Berry[2], and Jonathan Hirst[1]

[1]Mothers and Babies Research Centre, HMRI, University of Newcastle, NSW 2309; [2]Department of Paediatrics and Child Health, University of Otago, Wellington, New Zealand.
Julia.shaw@uon.edu.au

Background: Preterm birth is increasing in developed nations and is associated with the onset of neurodevelopmental and behavioural disorders later in life. Clinically, premature males are at risk of developing ADHD and aggressive type behaviours in childhood. The lack of suitable animal models of prematurity reduces our ability to treat and protect these vulnerable neonates. The precocial nature and the maturity of neurodevelopment at the time of birth in the guinea pig means that it may be a suitable model to study the development of behavioural disorders in juvenility of ex-premature males.

Aims/Hypothesis: This project aimed to characterise the behavioural profile of male juvenile guinea pigs following preterm birth.

Methods: Guinea pig neonates were delivered preterm by induction of labour (GA62) or delivered spontaneously at term (GA69). Open field and social interaction behavioural testing was carried out at juvenility (PND25) and analysed by ANY-maze tracking software.

Results: Preterm males travelled greater distances than term counterparts in the open field ($p=0.041$). Interestingly, preterm males exhibited more hyperactivity-type behaviour than term males as they had significantly more entries, more time spent, and more distance travelled in the inner zone ($p=0.05$, $p=0.042$, and $p=0.046$). Furthermore, preterm males also approached, sniffed and attempted to bite familiar cage mates more than term males, indicating increased aggressive behaviour.

Conclusions: Preterm male juvenile guinea pigs exhibit a more hyperactive, investigative and possibly aggressive behavioural profile than term male juvenile guinea pigs. These observations are in line with the literature as hyperactivity and aggressive type behaviours are more common in males born preterm. The establishment of this clinically relevant model will now allow for the exploration of mechanisms and treatment options for these vulnerable offspring.

Session 3

Session 3: Chairs – David Todd and Beverly Muhlhausler			
2.30	**A14**	Kyungjoon Lim (ECR)	Trans-generational effect of maternal obesity on the programming of hypertension: altered leptin signalling pathway in the central nervous system
2.45	**A15**	Erandi Hewawasam (E)	miRNA biomarkers for early prediction of adverse pregnancy outcomes in obese pregnant women
3.00	**A16**	Sabiha Chowdhury (E)	Impact of maternal obesity in rat offspring kidney
3.15	**A17**	Jack Darby (E)	The heart is resistant to increased glucose availability in late gestation
3.30	***A18**	Dayana Mahizir (E)	High fat diet exacerbates glucose intolerance in pregnant females born small
3:38	***A19**	Jessica Gugusheff (ECR)	A maternal 'junk food' diet alters mu-opioid receptor mRNA expression in late but not early postnatal development in female offspring
3:46	**General discussion**		

Trans-generational effect of maternal obesity on the programming of hypertension: Altered leptin signalling pathway in the central nervous system

Kyungjoon Lim[1], Sandra L Burke[1], Geoffery A Head[1,2]

[1]Neuropharmacology, Baker IDI Heart & Diabetes Institute, Melbourne, Australia; [2]Dept of Pharmacology, Monash University, VIC 3800, Australia.
Joon.lim@bakeridi.edu.au

Background: The prevalence of obesity in women among child baring age is increasing and this has been parallel to the increase in obesity in general population around the world.

Aims/Hypothesis: We investigated the trans-generational 'programming' of leptin signalling in the central nervous system (CNS) to increase blood pressure (BP), heart rate (HR) and renal sympathetic nerve activity (RSNA) following a high fat diet (HFD) feeding in mothers.

Methods: Female New Zealand White rabbits were fed a high fat (13%) diet (mHFD) or a control diet (mCD) prior mating and during pregnancy. Kittens from mCD rabbits were subdivided and fed HFD for 10days (mCD10dHFD) at 15weeks of age. All rabbits received an intracerebroventricular (ICV) catheter into the lateral ventricle and a recording electrode on the left renal nerve. Experiments were conducted in conscious rabbits and BP, HR and RSNA was measured. Rabbits received an increasing dose of ICV Melanocortin receptor antagonist (SHU9119), α-Melanocortin stimulating hormone (αMSH) and a single dose of Leptin antagonist, Insulin antagonist and Ringer's.

Results: Baseline measurement of BP (75.7±2.3 and 79.5±3.2 mmHG), HR (184.1±4.6 and 177.3±4.8 beats) and RSNA (9.9±1.6 and 12.0±0.6 nu) were increased in mHFD and mCD10dHFD rabbits compared to mCD rabbits (68.1±0.7mmHg, 173±3.3beats and 5.2±0.7nu, respectively). ICV SHU9119 reduced BP (-6.2±0.6mmHg and -3.7±0.7mmHg) and RSNA (-1.5±0.2 nu and -1.5±0.3 nu) in mHFD and mCD10dHFD rabbits ($P<0.001$). αMSH injection increased BP (3.1±1.5mmHg and 4.8±1.2mmHg), HR (39.4±11.2 beats and 29.4±8.7 beats) and RSNA (5.4±2.2nu and 4.2±0.7nu) in both mHFD and mNFD10dHFD rabbits ($P<0.001$). Leptin antagonist reduced BP and RSNA only in mHFD rabbits (-2.1±0.5mmHg and -3±0.2 nu, respectively) and Insulin antagonist reduced BP only in mCD10dHFD rabbits (-8.0±1.0mmHg). Total % fat was increased (50%) in all rabbits that were exposed to HFD.

Conclusions: Obesity during pregnancy 'programs' leptin signalling pathway in the CNS of the offspring during development. Leptin via activation of melanocirtin pathway plays a key role in the CNS contributing to the pressor and tachycardic effects as well as renal sympathetic nerve activity in the pathophysiology of obesity.

miRNA biomarkers for early prediction of adverse pregnancy outcomes in obese pregnant women

Erandi Hewawasam[1], Carina Bertoldi Franco[1], Ryan Farr[2], Cameron Kos[1], Viviane Delghingaro-Augusto[1], Anand Hardikar[2] & Christopher Nolan[1]

[1]Endocrinology and Diabetes research group, Australian National University, The Canberra Hospital, ACT 2605; [2]Diabetes and Islet biology Group, Sydney University, NSW 2050.
erandi.hewawasam@adelaide.edu.au

Background: Maternal obesity increases the risk of fetal and neonatal complications, placing a significant burden on health services. microRNAs (miRNAs) are small, non-coding RNAs that can modify the post-transcriptional regulation of gene expression. Furthermore, placenta is a rich source of miRNAs that are detectable in maternal plasma. Thus, maternal plasma miRNAs are a potential source of novel biomarkers for pregnancies at risk in obese women.

Aims/Hypothesis: We hypothesised that miRNAs will be differentially released from placental tissue under nutrient-induced stress, such as might occur in diabetic and obese women. The aim, therefore, was to determine the differential release of miRNAs from human trophoblasts in primary cell culture subjected to various nutrient stresses.

Methods: Placentas were collected from healthy lean women undergoing elective Caesarean-section at term. Trophoblasts were harvested and established in primary culture, then incubated for 48h in media with 5.5 or 25 mM glucose, with or without fatty acids (FA) (0.25mM oleate:palmitate 1:1). The culture media were then collected and assessed for differentially released miRNA profiles using the OpenArray™ system. Twelve differentially released miRNAs, if also shown to be detectable in plasma from obese pregnant women, were selected for further validation by qPCR.

Results: Of the ~754 miRNAs assessed on the OpenArray™ system, 37 miRNAs were differentially released into the cell culture media (29 increased and 8 decreased more than 2.5 fold) in the high glucose plus FA compared to the low glucose without FA condition. Importantly, 34 of the 37 differentially released miRNAs were detectable in maternal plasma collected from obese pregnant women at 26 weeks of pregnancy. qPCR validation of the selected 12 miRNAs showed that elevated glucose generally increased, whereas the presence of FA generally decreased, their release into the culture media.

Conclusions: This research supports the concept that maternal placental miRNAs may serve as biomarkers to detect pregnancies at risk in obese mothers.

Impact of maternal obesity in rat offspring kidney

Sabiha Sultana Chowdhury, Hasnah Bahari, Vanni Caruso, Christopher A. Maloney & Margaret J. Morris

Department of Pharmacology, School of Medical Sciences, University of New South Wales, Sydney, New South Wales, Australia.
sabiha.chowdhury@unsw.edu.au

Background: Obesity is associated with diabetes and hypertension, two common risk factors for chronic kidney disease. In parallel with diabetes and obesity, chronic kidney disease is increasing at an alarming rate. Evidence suggests that maternal obesity programs offspring disease risk, making them more prone to obesity, diabetes, and hypertension but the link between maternal obesity and offspring kidney disease remains unclear.

Aim: The impact of maternal obesity on offspring kidney and whether it is worsened by an unhealthy post-weaning diet was studied in a rat model. Exercise is known to have beneficial effects on the kidney thus siblings either remained sedentary or were given access to a running wheel.

Methods: Sprague Dawley female rats were fed either chow diet or high-fat diet (HFD) for 5 weeks before mating. Female offspring were weaned onto either chow diet or HFD and after 7 weeks, half of the offspring were exercised (running wheel) for 5 weeks. The offspring were killed at 15 weeks; kidneys were frozen for protein and mRNA extraction. The body weight, kidney mass, and triglyceride levels were measured.

Results: A significant maternal and post weaning diet effect on final body weight of offspring was observed and exercise was found to reduce body weight (Bahari et al. 2013). Maternal diet was associated with a significant increase in kidney triglyceride accumulation; chow fed offspring of obese mums showed a 91% increase in triglyceride content in their kidney ($P < 0.01$). Post-weaning HFD consumption also increased kidney triglyceride content by 37% and 20% in offspring of lean and obese mums, respectively ($P < 0.01$). However exercise had no impact on kidney triglyceride accumulation.

Conclusions: Our data suggest that maternal diet has a greater effect than current HFD consumption on triglyceride accumulation in offspring kidney. Voluntary exercise in offspring did not reduce this triglyceride content in kidney. Current work is examining gene expression in an attempt to find out the underlying mechanism.

Reference:

Bahari, H., Caruso, V. and Morris, M. J. (2013) Late-Onset Exercise in Female Rat Offspring Ameliorates the Detrimental Metabolic Impact of Maternal Obesity. Endocrinology, 154(10), pp. 3610-3621.

The heart is resistant to increased glucose availability in late gestation

Jack R Darby, Song Zhang, I Caroline McMillen and Janna L Morrison

Early Origins of Adult Health Research Group, Sansom Institute for Health Research, University of South Australia, SA 5000, Australia.
Jack.Darby@mymail.unisa.edu.au

Background: Maternal obesity predisposes offspring to a greater risk of developing cardiovascular disease in adult life. The mechanisms by which this occurs are not fully understood. Pregnancy in obese women is often associated with the onset of gestational diabetes, resulting in a fetus that is hyperglycaemic and hyperinsulinaemic in late gestation. In the adult, the onset of diabetic cardiomyopathy occurs under similar conditions and is characterised by cardiomyocyte hypertrophy, apoptosis and myocardial fibrosis. The effect that these conditions have on the fetal heart is not known.

Aims/Hypothesis: The present study evaluated the effects of fetal glucose infusion in late gestation on the mRNA and protein abundance of molecules involved in the regulation of cardiomyocyte growth and metabolism. We hypothesize that high glucose and insulin concentrations in late gestation would result in a cardiac phenotype similar to that of an adult with diabetic cardiomyopathy.

Methods: Either saline or glucose was infused into sheep fetuses from 130 to 140 days (d) gestation (term=150d). mRNA (qRT-PCR) and protein (Western blot) expression of molecules involved in the regulation of cardiac development and metabolism were determined

Results: Intrafetal glucose infusion did not alter the expression of molecules within the signalling pathways that regulate hypertrophy, apoptosis or fibrosis in the fetal heart. *GLUT-1* mRNA expression was decreased but this change did not correspond with a decrease in GLUT-1 protein abundance. Glucose infusion increased the mRNA expression of both *PPARα* and *PPARγ*. However, there was no change to PPAR transcription cofactors or mRNA expression of molecules with PPAR response elements.

Conclusions: Increased plasma glucose and insulin concentrations in late gestation did not result in a cardiac phenotype similar to that of an adult with diabetic cardiomyopathy. The present study shows that the fetal heart in late gestation is relatively resilient to increased glucose supply.

High fat diet exacerbates glucose intolerance in pregnant females born small

Dayana Mahizir[1], Kristina Anevska[1], Andrew J. Jefferies[1], Glenn D. Wadley[2], Deanne H Hryciw[1], Karen M. Moritz[3] & Mary E Wlodek[1]

[1]Department of Physiology, The University of Melbourne, Parkville, Victoria, Australia; [2]Centre for Physical Activity and Nutrition Research, School of Exercise and Nutrition Sciences, Deakin University, Burwood, Victoria, Australia; [3]School of Biomedical Sciences, University of Queensland, St. Lucia, Queensland, Australia.
nmahizir@student.unimelb.edu.au

Background: Intrauterine growth restriction programs adult metabolic disease and the phenotypes are exacerbated with "second hits" such as pregnancy and obesity in women born small. We have recently reported that the physiological challenge of pregnancy unmasks glucose intolerance in females born small. Importantly, growth restricted females are at an increased risk of developing obesity. Therefore, it is likely that maternal obesity may exacerbate the pregnancy complications in women born small.

Aims/Hypothesis: This study will determine if the adverse metabolic adaptations to pregnancy in F1 rats that were born small are exacerbated with high fat diet.

Methods: Uteroplacental insufficiency was induced by bilateral uterine artery ligation (Restricted) or sham (Control) surgery on E18 in Wistar-Kyoto female rats. Female offspring were fed a chow or high fat (43% kcals from fat) diet from 5 weeks of age to mating (20 weeks) and throughout the pregnancy. Glucose tolerance test was performed (E18) and their dorsal fat weights and plasma leptin concentrations were measured at E20.

Results: Restricted and Control female rats that were exposed to a high fat diet were significantly heavier with 27% and 34% more dorsal fat than females on a chow diet. Similarly, plasma leptin concentrations were higher in Restricted (+59%) and Control (+30%) female rats on a high fat diet compared to females on a chow diet. High fat diet exacerbated the pre-existing glucose intolerance (+15% AUC) in pregnant females born small compared to growth-restricted females on a chow diet.

Conclusions: Females born small who consume a high fat diet are more susceptible to increased adiposity and exacerbated glucose intolerance during pregnancy with a phenotype similar to gestational diabetes.

A maternal 'junk food' diet alters mu-opioid receptor mRNA expression in late but not early postnatal development in female offspring

Jessica Gugusheff[1], Iain Clarke[2], Zhi Yi Ong[3] & Beverly Muhlhausler[1]

[1]FoodPlusResearch Group, School of Agriculture, Food and Wine, University of Adelaide, SA, 5000, Australia; [2]Department of Physiology, Monash University, VIC, Australia; [3]Department of Psychology, University of Pennsylvania, Philadelphia, Pennsylvania, USA.
jessica.gugusheff@adelaide.edu.au

Background: Previous studies have demonstrated that exposure to a maternal junk food diet during the perinatal period reduces the sensitivity of the opioid pathway and increases the preference for palatable diets in juvenile and adult offspring. The present study aims to further explore the mechanisms behind the desensitization of the opioid pathway in the offspring of junk food fed mothers, by determining how early in development changes in mu-opioid receptor expression occur.

Aims/Hypothesis: To compare mu-opioid receptor mRNA expression during early (at birth and week 1) and late (week 3 and 4) postnatal development in the offspring of dams feed either a control or junk food diet.

Methods: 10 Albino Wistar female rats were provided with either a junk food (JF, n=5), or standard chow diet (Control, n=5), during pregnancy and lactation. Brain tissue was collected from the offspring at birth, week1, week 3 and week 4, snap frozen and sagittal cryostat section cut to include both the nucleus accumbens (NAc) and the ventral tegmental area (VTA). Radioactive *in situ* hybridisation was then used to determine the mRNA expression of the mu-opioid receptor in these brain areas.

Results: The number of cells with mu-opioid receptor mRNA expression in the VTA of female JF offspring was 32% (week 3) and 57% (week 4) lower than in their Control counterparts ($P<0.05$), this difference was not evident in early development. We also observed a significant decrease in the number of cells with mu-opioid receptor expression in the NAc in late postnatal development compared to early postnatal development ($P<0.05$), independent of maternal diet in both male and female offspring.

Conclusions: These findings suggest that exposure to a maternal junk food diet lowers mu-opioid receptor expression in late but not early postnatal development and that this effect is sex specific. The outcomes of this study provide valuable additional information on a potential mechanism behind the programming of food preferences.

Session 4

Session 4: Chairs – Nicolette Hodyl and Jeff Craig			
4.30	**A20**	Jacinta Kalisch-Smith (E)	Peri-conceptional alcohol exposure does not alter pre-implantation phenotypes but can affect trophoblast differentiation in culture
4.45	**A21**	Diana Zanfirache (E)	Behavioural correlates of periconceptional ethanol exposure in aged offspring
5.00	**A22**	Lisa Yamada (L)	Nicotine exposure in early pregnancy: mid-gestational growth restriction correlates with differential mRNA and microRNA expression
5:15	**A23**	Richard Schlegel (L)	The effect of maternal hypomagnesaemia on fetal development and the programming of adult disease
5:30	***A24**	Lisa Akison (ECR)	Prenatal alcohol exposure and female reproductive health – potential impacts on the fertile life span of offspring
5:38	***A25**	Jeffrey Craig	Do low, moderate and high patterns of prenatal alcohol consumption influence placental epigenetics?
5.46	**General discussion**		

Peri-conceptional alcohol exposure does not alter pre-implantation phenotypes but can affect trophoblast differentiation in culture

Kalisch-Smith, J.I., Pantaleon, M., Simmons, D.G., Moritz, K.M

School of Biomedical Sciences, University of Queensland, QLD 4072, Australia.
jacinta.kalischsmith@uqconnect.edu.au

Background: Ethanol consumption is widespread among pregnant women in Australia, particularly prior to pregnancy recognition. We have shown previously that maternal periconceptional alcohol (PC-EtOH) exposure can cause foetal growth restriction and sex-specific changes to placental morphology in late gestation. However, analysis of trophectoderm (TE) and inner cell mass (ICM) derivatives after PC-EtOH exposure currently remains unknown.

Aims/Hypothesis: This study utilises both *in vivo* and *in vitro* methodologies to determine the effects of ethanol on the pre-implantation embryo and downstream cell types to both the embryo proper, and the placenta.

Methods: Sprague Dawley dams were administered 12.5% v/v ethanol or a control diet from 4 days prior (E-4) to 4 days after conception (E4) in a liquid diet. Pre-implantation embryos were assessed for embryonic stage, cell number and allocation to the ICM or TE at E5. A subset of E5 embryos were cultured *in vitro* for 6 days and measured for outgrowth area. To determine whether ethanol can directly affect the differentiation of placental trophoblast stem (TS) cells, two mouse lines, one of each sex, were cultured *in vitro* for 6 days (n=3 per treatment), in 0%, 0.2% or 1% ethanol and assayed for lineage-restricted trophoblast subtype markers. RNA was extracted for q-PCR and the expression of genes specific to the labyrinth (*Ctsq, Syna*) and junctional zones (*Tpbpa, Prl7a2, Prl7b1, Prl2c1, Prl3d1*) were analysed.

Results: Preliminary data has found no differences in pre-implantation developmental stage, total cell number, differential cell count, or outgrowth area (6-11 embryos per treatment) after *in vivo* PC-EtOH exposure. Both TS cell lines showed dose-dependent reductions in the expression of markers for the labyrinth (*Syna,* $P<0.01$) and the junctional zone (*Prl7b1, Prl7a2,* $P<0.01$). Curiously, the female line also showed a reduction in junctional zone and their progenitor marker *Tpbpa* ($P<0.01$). No changes were found for the remaining markers.

Conclusions: The observed reductions in gene expression suggest alcohol exposure may either delay TS cell differentiation or alter cell allocation to specific lineages. Analysis of TE stem cell markers (*Cdx2, Eomes, Esrrb*) after 2 days of culture will examine whether precocious differentiation can occur. Expression profiles of blastocyst outgrowths for TE and ICM (*Oct4, Gata6, Nanog*) markers will support whether altered differentiation occurs *in vivo*. The observed alterations may contribute to altered placental development, foetal growth restriction and programming of adult disease.

Behavioural correlates of periconceptional ethanol exposure in aged offspring

Karen M Moritz, Carlie Cullen & Diana Zanfirache

School of Biomedical Sciences, The University of Queensland, Brisbane, Queensland, 4072.
Diana.zanfirache@uqconnect.edu.au

Background: Maternal alcohol consumption prior to recognition of pregnancy is a common social practice. This time frame is referred to as the periconceptional period (PC) and has been recognised as a critical developmental window as the developing embryo is highly responsive to its environment. Very little is known about the impact of PC alcohol exposure on long term cognitive function. However, long lasting changes to the epigenome are proposed as a mechanism through which periconceptional exposure may lead to impaired long term cognitive dysfunction.

Aims/Hypothesis: The aim of this study was to examine the effect of PC ethanol (PC EtOH) exposure on long term cognitive function; including memory and anxiety.

We hypothesised that PC EtOH would lead to behavioural changes in aged animals particularly deficits in spatial memory and anxiety. We also hypothesised there would be changes in gene expression of regulators of the epigenome: in particular the DNA methyltransferases (DNMT1, DNMT3a) and the histone modifiers (HDAC-2)

Methods: Female Sprague-Dawley rats were exposed to a liquid diet containing ethanol (EtOH) (12.5% vol;vol) or a control diet during the PC period (from embryonic day (E)-4 to E4). Male (n=8, Control; n=8 EtOH) and female (n=9 Control; n=7 EtOH) aged offspring were put through a battery of behavioural tests to assess aspects of anxiety like behaviour and spatial memory. Brain tissue (hippocampus) was collected and examined for gene expression levels of epigenetic modifiers.

Results: PC EtOH exposure resulted in a significant ($p=0.0001$) increase in directed exploring/head dipping behaviour during holeboard testing. However, PC EtOH exposure did not result in an anxiety like phenotype (elevated plus maze). Interestingly, the female PC EtOH rats showed short term spatial memory impairment displaying a significant decrease ($p<0.04$) in the percentage (%) of time spent in the novel arm of the Y maze.

Conclusions: Exposure to PC EtOH did not lead to anxiety like behaviour in the aged offspring. The decrease in the % of time spent in the novel arm indicates exposure to PC EtOH may induce sex specific impairments in spatial memory with females more affected than males. Preliminary gene expression studies suggest there may be changes in expression of epigenetic modifiers in the hippocampus.

Nicotine exposure in early pregnancy: mid-gestational growth restriction correlates with differential mRNA and microRNA expression

Lisa Yamada[1,2], Elizabeth Mason[2], Christine Wells[2], Suyinn Chong[1,2]

[1]Epigenetics Group, Mater Research Institute – The University of Queensland, Translational Research Institute, QLD 4102, Australia; [2]The University of Queensland, QLD 4072, Australia.
Lisa.Yamada@mater.uq.edu.au

Background: Prenatal nicotine exposure in experimental animal models is associated with growth restriction, morphological malformations and postnatal behavioural changes in the offspring. Studies to date have primarily focused on exposures which cover the entirety of gestation. However, the effect of nicotine exposure in early gestation alone is poorly characterised.

Aims/Hypothesis: This study aimed to determine what phenotypic and transcriptional effects moderate nicotine exposure during early gestation has on the mid-gestational embryo.

Methods: Pregnant C57BL/6J mice were provided with ad libitum access to 100 µg/ml nicotine from 0.5 to 8.5 dpc (equivalent to the first 3-4 weeks of human gestation). Resultant embryos were assayed at 9.5 dpc for crown-rump length and somite number, genome-wide gene expression (Illumina MouseWG-6 v2.0 Expression BeadChip), and microRNA expression (TaqMan miRNA Array).

Results: A reduction in crown-rump length was observed in nicotine-exposed embryos when compared to control embryos ($p<0.001$). In the same embryos, somite numbers were unchanged between groups, suggesting this growth restriction is not a result of developmental delay. Of 105 differentially expressed genes, 21 were correlated to a measure of embryonic size (crown-rump length normalised to somite count). Many of these genes have annotated roles in growth and metabolism. 14 miRNAs were also differentially expressed, all of which have predicted (DIANA miRPath v2.0) targets in the insulin signalling pathway.

Conclusions: Maternal nicotine consumption, even when restricted to early pregnancy, can elicit growth restriction with correlated transcriptional consequences in the mouse. Further, these transcriptional changes indicate a possible metabolic phenotype.

The effect of maternal hypomagnesaemia on fetal development and the programming of adult disease

Richard Schlegel, James Cuffe, Jereme Spiers, Carlie Cullen, Karen Moritz & Tamara Paravicini

School of Biomedical Sciences, The University of Queensland, QLD 4072, Australia.
richard.schlegel@uqconnect.edu.au

Background: Magnesium is essential for fetal development and the maintenance of normal cellular processes within the body. Although it is well characterized that maternal nutrient deficiencies can adversely affect fetal development and offspring health, few studies have explored the implications of maternal Mg deficiency on fetal development and programmed outcomes in offspring.

Aims/Hypothesis: To examine how Mg deficiency during pregnancy affects fetal and placental development, and the consequences for cardiovascular, renal, and neurological outcomes in adult offspring.

Methods: Female CD1 mice received a control (0.2% Mg), or Mg-deficient (0.02% Mg) diet for 4 weeks prior to mating and throughout pregnancy until weaning (PN21). Foetuses and placentas were collected at E18.5. Blood pressure (radiotelemetry), 24 h electrolyte excretion (metabolic cages), and anxiety-like behaviours (Suok and elevated plus maze) were assessed in a separate cohort of offspring at 6 months.

Results: Maternal hypomagnesaemia caused fetal loss and modest growth restriction ($P<0.05$). Placentas from Mg-deficient dams showed increased glycogen deposition ($P<0.05$) and reduced spongiotrophoblast cross-sectional area ($P<0.05$). At 6 months of age, Mg-deficient offspring showed minor changes in renal function with reduced Mg excretion (males only) and increased urinary flow (both sexes) ($P<0.05$), but no change in nephron number. Offspring showed no differences in blood pressure or heart rate. Mg-deficient male offspring exhibited reduced head dips (control, 101 ± 5; Mg-deficient, 61 ± 5; $P<0.001$) during the Suok test, and reduced open arm exploration (control, 20% ± 4%; Mg-deficient, 8% ± 3%; $P<0.05$) during the elevated plus maze. Female offspring showed no differences in behaviour. Mg deficiency altered hippocampal expression of NMDA receptor subunits, increasing the NR2B:NR2A ratio (both sexes) and reducing NR1 levels (males only, $P<0.05$).

Conclusions: Dietary Mg intake plays a pivotal role in fetal health and adult outcomes. Maternal hypomagnesaemia led to altered placental morphology, growth restriction, and fetal loss. These changes were associated with neurological deficits and minor changes in renal function in the adult offspring, but did not affect cardiovascular physiology.

Prenatal alcohol exposure and female reproductive health – potential impacts on the fertile life span of offspring

Lisa Akison & Karen Moritz

School of Biomedical Sciences, University of Queensland, Brisbane QLD 4072, Australia.
l.akison@uq.edu.au

Background: Alcohol consumption amongst women of reproductive age is widespread in Australia (~80%). Given that ~50% of all pregnancies are unplanned and that >40% of women continue to drink alcohol following pregnancy diagnosis, this potentially exposures the early embryo and fetus to alcohol during development. The Moritz laboratory has developed two rat models of prenatal alcohol exposure designed to mimic common human drinking patterns during pregnancy. These result in fetal growth deficits and adult onset metabolic, cardiovascular and renal dysfunction in offspring. Interestingly, kidney development is perturbed, resulting in reduced kidney weight and life-time nephron endowment. The ovaries, like the kidneys, develop prenatally in mammals, with the life-time supply of oocytes established and arrested in an immature state in primordial follicles. These form that individual's 'ovarian reserve', with only a small number destined to grow and eventually capable of ovulating a mature oocyte that can be fertilised. Importantly, in humans, there appears to be a link between the initial ovarian reserve at birth and age at menopause, with variability in the former translating to a dramatic range in the latter (~40-60 years). *Given that women are choosing to have children later in life, it is critical to understand what impacts the initial establishment of oocyte number.*

Aims/Hypothesis: Given the common origin of the kidneys and gonads during development and similarities between the prenatal establishment of nephron endowment in the kidney and the primordial follicle pool in the ovary, we hypothesise that ovarian development is also perturbed in females of alcohol exposed mothers. We aim to: 1) compare initial primordial follicle numbers at PN10 in alcohol exposed and control dams; 2) compare breeding performance and ovarian folliculogenesis/reserve of young adult offspring from alcohol exposed and control dams; and 3) examine the impact of ageing as a '2nd Hit' to ovarian reserve and fertility in offspring from alcohol exposed and control dams.

Methods: We will use our rat models of periconceptional (12% v/v EtOH, E-4 to E4) or low dose chronic (6% v/v EtOH, E1 to birth) alcohol exposure using isocaloric, nutritionally balanced liquid diets with no EtOH controls. Stereological analysis of ovaries will be conducted at 3 time points to quantify primordial follicle numbers (at PN10) and subsequent stages of follicular development (at 8 weeks and 12 months). In both adult cohorts, ovulation rate and oocyte quality will be measured. In separate groups of animals, following mating with males, litter size will be assessed in at least 3 consecutive litters per female.

Outcomes and Significance: This study will address fundamental questions about the effect of prenatal alcohol exposure on fetal ovarian development. It will also examine the potential for developmental programming of ovarian reserve and ovarian function in adulthood.

Do low, moderate and high patterns of prenatal alcohol consumption influence placental epigenetics?

Jeffrey M Craig, Evelyne Muggli, Jane Halliday

Murdoch Childrens Research Institute, Royal Children's Hospital, Parkville, Australia.
Jeff.craig@mcri.edu.au

Background: The Asking QUestions about Alcohol in pregnancy (AQUA) study has used a novel method of prenatal alcohol classification that incorporates dose, pattern, and timing of alcohol exposure. It has categorised exposure levels ranging from total abstinence to low, moderate and heavy levels of drinking from the first to the third trimester. This presentation reports on a pilot study of analysis of the epigenetic mark of DNA methylation in placental DNA from 200 AQUA pregnancies.

Aims/Hypothesis: The aims of the AQUA study are: (1) to develop a multifactorial risk model for the effects of prenatal alcohol consumption on the health and development of offspring; (2) to provide evidence-based advice to the public and health professionals on the consequences of low, moderate and heavy levels of prenatal alcohol consumption. We hypothesise that epigenetics is a mediator of prenatal alcohol exposure on postnatal risk of compromised neurodevelopment.

Methods: Two hundred placental DNA samples were analysed for DNA methylation at a global level, using proxies of Alu and LINE-1 interspersed repeats and in two imprinted gene loci, IGF2/H19 and DLK1/DIO3, using Sequenom MassArray EpiTyper. A subset of samples from abstinent and moderate to heavy alcohol exposures were examined across the whole genome using the Illumina Infinium HM450 BeadArray platform, which measures DNA methylation at almost half a million genomic locations of functional importance.

Results: Preliminary data analysis found a wide variation in epigenetic state throughout all placental samples, but no correlation with alcohol exposure was observed. Factors such as smoking and folate intake had assay-dependent effects on DNA methylation within the placenta. Multiple regression analyses are currently underway.

Conclusions: No direct effects of prenatal alcohol exposure were found on the epigenetic mark of DNA methylation in placentae from women consuming low to moderate/high levels of alcohol in the prenatal period. Consideration of co-factors may assist in clarifying the relationship between alcohol exposure and epigenetics at birth.

Session 5

Session 5: Chairs – Ian Wright and Yvonne Eiby			
9:15	**A26**	Yvonne Eiby	Low cardiac preload is detrimental to cardiovascular function and cerebral blood flow in preterm piglets
9:30	**A27**	Joe Smolich	Discordance between phasic changes in the aorto-pulmonary pressure difference and ductal blood flow during the preterm birth transition
9:45	**A28**	Rebecca Dyson (ECR)	Vascular dysfunction in the newborn: contribution of the gasotransmitters (and their interactions)
10:00	**A29**	Stacey Vranas (E)	The effects of intrauterine growth restriction on adult heart structure in an ovine model
10:15	**A30**	Paul Lombardo (L)	An evaluation of the effects of moderate preterm birth on the heart and major arteries of male lambs from birth to twelve months of age using serial ultrasound imaging
10:30	***A31**	Corinna Binder-Heschl	Effect of prenatal caffeine infusion on the newborn cardiovascular system at birth
10:38	**General discussion**		

Low cardiac preload is detrimental to cardiovascular function and cerebral blood flow in preterm piglets

Nicole Shrimpton[1], Yvonne Eiby[1], Eugenie Lumbers[1,2], Paul Colditz[1], Ian Wright[3], Greg Duncombe[1] & Barbara Lingwood[1]

[1]Perinatal Research Centre, UQ Centre for Clinical Research, The University of Queensland, QLD 4029, Australia; [2]Hunter Medical Research Centre, University of Newcastle, NSW 2308, Australia; [3]Illawarra Health and Medical Research Institute, University of Wollongong, NSW 2522, Australia.
y.eiby@uq.edu.au

Background: Prevention of brain injury in children born very preterm requires optimal cardiovascular support on the first day of life. Cardiac contractility, hence cardiac output, is dependent on preload (filling pressure) but very preterm infants are likely to have low preload due to excessive vasodilatation and reduced circulating blood volume.

Aims/Hypothesis: To determine the effect of reduced preload on cardiovascular function, cerebral blood flow and response to inotropes in term and preterm piglets.

Methods: Piglets were delivered by caesarean section either preterm (97d/115d; similar to a baby born at 27wk gestation) or at term (114d/115d). Following resuscitation, piglets were maintained under standard neonatal intensive care conditions for up to 10h. Cardiac contractility (dP/dt max), cardiac output, arterial blood pressure, and cerebral blood flows were measured during baseline and then again during lower preload (induced by removal of 5-10% of blood volume), and following infusion of dopamine or dobutamine (10μg/kg/min then 20 μg/kg/min).

Results: In term piglets (n=24), 10% of the blood volume was removed (based on 80mL/kg BW) and this led to a 27-36% reduction in cardiac contractility, cardiac output and arterial blood pressure. There was no significant reduction in cerebral blood flow in term piglets. In preterm piglets (n=24), 5-7.5% of blood volume was removed and this led to a 55-65% reduction in cardiac contractility, cardiac output and arterial blood pressure. There was a 75% reduction in cerebral blood flow. Cerebral blood flow was not restored by dopamine or dobutamine infusion.

Conclusions: Term piglets compensate effectively for low preload and cerebral blood flow is maintained. In contrast, in preterm piglets, cerebral blood flow falls to 25% of baseline levels. These findings suggest that new treatments designed to maintain preload are imperative to protecting the preterm brain.

Discordance between phasic changes in the aorto-pulmonary pressure difference and ductal blood flow during the preterm birth transition

Joe Smolich,[1,2] Kelly Kenna[1] & Jonathan Mynard[1,2]

[1]Heart Research Group, Murdoch Childrens Research Institute; [2]Department of Paediatrics, University of Melbourne, VIC 3052, Australia.
Joe.smolich@mcri.edu.au

Background: Mean blood flow across the ductus arteriosus is right-to-left in the fetus but rapidly reverses to left-to-right within minutes of birth. A difference in mean blood pressure also exists between the pulmonary (PT) and aortic trunk (AoT), which is positive in the fetus, and becomes negative within minutes after birth. Based on the foregoing patterns, it is widely presumed that changes in the PT-to-AoT pressure difference result in perinatal alterations of ductal flow. However, as both the PT-to-AoT pressure difference and ductal flow have distinct phasic (i.e. systolic and diastolic) components, we evaluated whether perinatal phasic changes in the PT-to-AoT pressure difference and ductal flow paralleled one another in the birth transition.

Aims: To compare temporal changes in systolic and diastolic components of the PT-to-AoT pressure difference and ductal flow in the preterm birth transition.

Methods: Nine anaesthetised preterm fetal lambs (weight 3.4±0.4 kg, age 126-129 days, term =147 days) were instrumented with 1) a 8-10mm transit-time flow probe on the ductus arteriosus, 2) AoT and PT fluid-filled catheters and 3) AoT and PT high fidelity micromanometer catheters to accurately measure the PT-to-AoT pressure difference. Haemodynamic data was recorded in the fetal state and, following cord clamping and the start of mechanical ventilation, at 1, 2, 3, 4, 6, 8, 10 and 15 min, and at 15 min intervals thereafter to 60 min after birth.

Results: Both the mean PT-to-AoT pressure difference (2.4±1.3 mmHg) and ductal blood flow (519±164 ml/min) in fetuses fell to zero within 2 min after birth ($P<0.001$), and were negative at 6 min and beyond, in association with marked changes in profile morphology. However, in fetuses, diastolic PT-to-AoT pressure difference was statistically zero (0.3±0.4 mmHg) even though diastolic ductal flow (95±46 ml/min) comprised ~20% of mean flow. Further, after birth, the systolic PT-to-AoT pressure difference was negative at >6 min, despite a near-zero systolic ductal flow.

Conclusions: The discordance between perinatal phasic changes in the PT-to-AoT pressure difference and ductal flow imply that changes in ductal flow patterns at birth are not simply related to alterations in the PT-to-AoT pressure difference.

Vascular dysfunction in the newborn: contribution of the gasotransmitters (and their interactions)

Rebecca Dyson[1,2], Hannah Palliser[2], Kelsee Shepherd[2], Ahmad Abbas[1], Megan Kelly[1], Joanna Latter[2], Grazyna Chwatko[3], Rafal Glowacki[3], Ni Xin[4], Ian Wright[1,2]

[1]Graduate School of Medicine, University of Wollongong, NSW 2522, Australia; [2]Mothers & Babies Research Centre, University of Newcastle, NSW 2305, Australia; [3]Department of Environmental Chemistry, University of Lodz, 90-236, Poland; [4]Department of Physiology, Second Military Medical University, Shanghai, China.
rdyson@uow.edu.au

Background: The gasotransmitters nitric oxide (NO), carbon monoxide (CO), and hydrogen sulphide (H_2S) drive microvascular dysregulation in preterm neonates. However, we do not fully understand what drives the production of these molecules, or their combined effects and interactions.

Aims/Hypothesis: Aim 1. Measure the levels of all three gasotransmitters, characterise their interrelationships and assess their combined effects in our human population. Aim 2. Characterise the contribution of key pathways (CO produced through Heme Oxygenase (HO) and H_2S produced through cystathionine-γ-lyase (CSE)) using an animal model.

Methods: *Human Studies* Structural equation modelling was used to examine the combination of gasotransmitter effects and derive a theoretical model of their interactions. *Guinea Pig Studies* Skin was collected fetally and at 10hr and 24hr postnatal age. Real-time H_2S production was assessed using a microrespiration system. HO levels were quantified by western blot.

Results: In male preterm neonates, increases in NO are associated with increases in H_2S and microvascular blood flow; whilst in females, CO negated the effect of H_2S on vascular tone. In our animal model, HO-1 expression was highest in those at risk of microvascular dysfunction: unstable preterm males. H_2S production increased postnatally and CSE-dependent H_2S production correlated with microvascular blood flow (in males only).

Conclusions: We propose a hypothetical model of gasotransmitter-dependent vasodilatation in the preterm newborn. These findings suggest varying roles for and interactions of the three gasotransmitters – allowing for regional and temporal control of blood flow: in male preterm neonates increases in NO are associated with increases in H_2S and microvascular blood flow. The current work gives some insight into the mechanisms underlying sexual dimorphism in outcomes following preterm birth.

The effects of intrauterine growth restriction on adult heart structure in an ovine model

Stacey Vranas[1], Julie A Owens[2], Kathryn L Gatford[2], M Jane Black[1]

[1]Fetal & Neonatal Research Group, Department of Anatomy & Developmental Biology, Monash University, VIC 3800, Australia; [2]Robinson Research Institute, University of Adelaide SA 5005, Australia.
savra1@student.monash.edu

Background: Individuals who have experienced intrauterine growth restriction (IUGR) are at increased risk of developing cardiovascular disease in adulthood. This may be linked to a reduced complement of cardiomyocytes formed in the heart at the beginning of life, which if it persists into adulthood has the potential to reduce life-long functional reserve.

Aims/Hypothesis: We hypothesised that induction of IUGR, due to placental restriction (PR), would reduce cardiomyocyte number and nuclearity, and increase cardiomyocyte size, in adult sheep.

Method: IUGR was induced by surgical removal of placental implantation sites to restrict placental growth and function in primiparous Merino x Border Leicester ewes. Cardiomyocyte number was estimated in 12 month old Control (n=5) and PR (n=5) male offspring, using an optical disector/fractionator approach, and cardiomyocyte size and nuclearity were determined using confocal microscopy. Data were analysed using an unpaired student's t-test and linear regression analyses were also conducted. Statistical significance was accepted at $P<0.05$.

Results: PR reduced birth weight compared to controls ($P<0.007$). Cardiomyocyte number, cross sectional area, longitudinal area and nuclearity in the LV+S did not differ between control and PR sheep. Cardiomyocyte number in the LV+S of the adult heart correlated positively with birth weight and adult heart weight ($P=0.003$ and $P=0.006$ respectively).

Conclusions: Both the in utero environment and size of the heart in adulthood influences the complement of cardiomyocytes in the LV+S. PR did not, however, reduce cardiomyocyte size and nuclearity.

An evaluation of the effects of moderate preterm birth on the heart and major arteries of male lambs from birth to twelve months of age using serial ultrasound imaging

Paul Lombardo[1], Michal Schneider[1], Robert De Matteo[1], Richard Harding[1], Vivian Nguyen[1], *Graeme Polglase[2] and *M Jane Black[1]

[1]Monash University, Clayton, Australia; [2]Monash Institute of Medical Research, Clayton, Australia.
**Joint senior authors*
paul.lombardo@monash.edu

Background: Cardiovascular adaptations in response to preterm birth are poorly understood with males at an increased risk of developing cardiovascular disease in later life. Structural changes in the heart and major arteries may contribute to the development of cardiovascular disease.

Aim: Our aim was to compare the heart and major arteries of preterm and term male lambs from birth to twelve months postnatal age using ultrasound imaging.

Method: Moderate preterm birth was induced at 0.9 of term in ewes after administration of antenatal corticosteroids (n=13 singleton male lambs). A control group of eleven singleton male lambs was born at term (147±1 days gestational age). Ultrasound imaging was conducted on all lambs at two days, two weeks, three, six and twelve months postnatal age. The thickness of the left ventricular wall, short axis internal chamber diameter of the left ventricle at the level of the papillary muscles, diameters of the aortic root and main, right and left pulmonary arteries were recorded, corrected for bodyweight and compared using a two-way ANOVA.

Results: The relative thickness of the anterior and posterior wall of the left ventricle, interventricular septum and the diameters of the aortic root and pulmonary arteries were significantly greater in preterm lambs compared to controls at 2 days and 2 weeks after birth. These differences were absent at three, six and twelve months postnatal age.

Conclusions: Compared to controls, male lambs born moderately preterm demonstrate structural differences in the heart and major arteries in the immediate postnatal period that could contribute to cardiovascular disease in later life.

Effect of prenatal caffeine infusion on the newborn cardiovascular system at birth

Corinna Binder-Heschl[1,2], Kelly Crossley[1], Graeme Polglase[1], Douglas Blank[1,3], Valerie Zahra[1], Alison Moxham[1], Karyn Rodgers[1], Stuart Hooper[1]

[1]The Ritchie Centre, MIMR-PHI Institute of Medical Research, Clayton, Australia; [2]Medical University of Graz, Graz, Austria; [3]Newborn Research Centre, The Royal Women's Hospital, Melbourne, VIC, Australia.
stuart.hooper@monash.edu

Background: Caffeine is a commonly used drug in preterm infants with apnea, but there is little knowledge about the effects of caffeine on haemodynamic parameters, especially during transition period. Routinely caffeine (10mg/kg caffeine-base) is given to the infant hours after birth, but in view of caffeine's success, an early administration in the delivery room has been suggested. However, we hypothesise that if caffeine is given to the infant prior to birth, it will not only stimulate breathing, but will also stimulate the cardiovascular system, especially after clamping the cord.

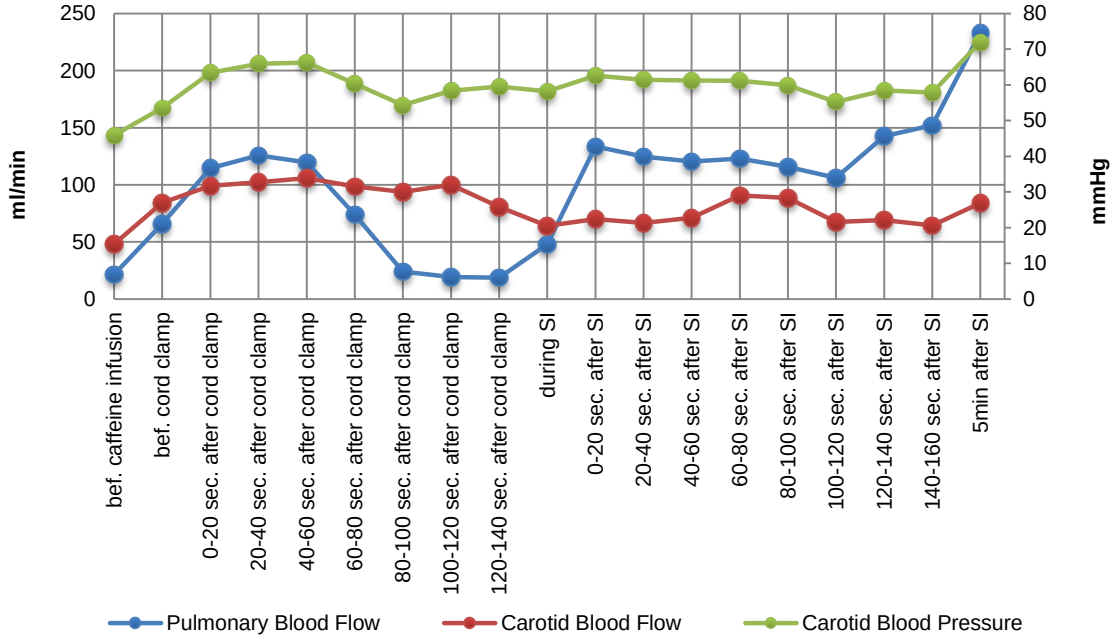
Aims: To determine the effects of prenatal caffeine administration on heart function and pulmonary blood flow during the transition of ventilated preterm lambs.

Methods: Lambs (≈125d of gestation) underwent surgery immediately before delivery by caesarean section, to instrument with catheters (carotid artery and jugular vein) and flow probes (pulmonary artery and carotid artery). Before clamping the cord lambs were intubated and a caffeine (10mg/kg) or saline infusion was given to the ewe and lamb via a venous line. After clamping the cord and a 2-minute observation period lambs received a sustained inflation (SI) (35 cm H_2O for 30 seconds) followed by ventilation for 30 minutes (target tidal volume of 6-8ml/kg).

Preliminary data: At this time point we investigated the pulmonary/carotid blood flow, the carotid blood pressure and the heart rate of two lambs after receiving prenatal caffeine infusion (Figure 1).

Conclusions: Preliminary data suggests that heart rate, pulmonary and carotid blood flow and carotid blood pressure increase immediately after prenatal caffeine infusion. Comparison to control lambs will be presented.

Figure 1: Pulmonary/ carotid blood flow and carotid blood pressure of a preterm lamb (125d GA) after receiving prenatal caffeine infusion.



Session 6

Session 6: Chairs – Jon Hirst and Foula Sozo			
11:25	**A32**	Jia Yin Soo (E)	Inflammatory and profibrotic markers in the lung of growth restricted offspring before and after birth
11:40	**A33**	Vicki Clifton	Identification of seven different isoforms of the glucocorticoid receptor in Guinea Pig placenta: relationship to preterm delivery, sex and betamethasone exposure
11:55	**A34**	Sandra Orgeig	Increased prolyl hydroxylase and decreased glucocorticoid receptor are related to decreased surfactant protein in the lung of the chronically hypoxemic growth restricted sheep fetus
12:10	**A35**	Bennet Seow (L)	Comparative effects of endogenous and synthetic glucocorticoid steroids during mammalian lung development
12:25	**A36**	Kirsten McInerney (E)	Adrenal contribution to neurosteroidogenesis in the neonate: effect of preterm birth and progesterone replacement
12:40	***A37**	Tamás Zakár	DNA methylation controls corticotropin releasing hormone (CRH) expression in human trophoblast cells
12:48	**General discussion**		

Inflammatory and profibrotic markers in the lung of growth restricted offspring before and after birth

Jia Yin Soo, Sandra Orgeig, Erin V McGillick, Song Zhang, I Caroline McMillen, Janna L Morrison

Sansom Institute for Health Research, University of South Australia, Adelaide, Australia.
jia_yin.soo@mymail.unisa.edu.au

Background: Although there is ample epidemiological and experimental evidence that intrauterine growth restriction (IUGR) reduces lung function in later life, little is known about the molecular mechanisms involved in predisposing growth restricted infants to poor lung function in childhood. We have previously shown that there is a decrease in surfactant protein abundance at 130 day gestation, which is ameliorated by 21 days after birth. This is an interesting finding in light of the known respiratory compromise that persists in children that were born small. Since many chronic lung diseases, including asthma and COPD, involve an inflammatory aspect and remodelling of the lung architecture, we aimed to evaluate the effect of IUGR on expression of inflammatory cytokines and regulators of extracellular matrix (ECM) in the lungs before and after birth.

Methods: Placental restriction (PR), leading to growth restriction was induced in ewes using carunclectomy. Lungs were collected from fetuses at 130 days gestation (Control, n=9; PR, n=5) and lambs at 21 days after birth (control, n=9; PR, n=6). mRNA abundance of inflammatory cytokines and regulators of the ECM and protein abundance of molecules involved in the Stat-3 pathway were quantified.

Results: PR resulted in an increase in IκB-α mRNA expression in the PR fetus and lamb. There was a significant increase in mRNA expression of IL-6 in the PR fetuses but not in PR lambs. Furthermore, there was a decrease in mRNA expression of macrophage inflammatory protein-1β in PR lambs, but no significant difference between control and PR fetuses. We also found that PR decreased mRNA expression of Matrix metallopeptidase-9 in the lungs of both fetuses and lambs. PR had no effect on Stat-3, phospho-Stat-3 or SOCS-3 protein expression at either age.

Conclusions: This study suggests that PR may lead to a dysregulation of inflammatory mediators and regulators of the ECM in the lungs, which may contribute to an increased risk of chronic lung disease in later life.

Identification of seven different isoforms of the glucocorticoid receptor in Guinea Pig placenta: relationship to preterm delivery, sex and betamethasone exposure

Vicki L Clifton[1], Zarqa Saif[1], Rebecca Dyson[2,3], Hannah Pallister[2], Ian MR Wright[2,3], Nick Lu[4]

[1]Robinson Research Institute, School of Paediatrics and Reproductive Health, University of Adelaide, Adelaide, SA; [2]Mothers and Babies Research Centre; Hunter Medical Research Institute, New Lambton Heights, NSW, 2305, Australia; [3]Discipline of Paediatrics and Child Health, School of Medicine and Public Health, University of Newcastle, Callaghan, NSW, 2308, Australia.
vicki.clifton@adelaide.edu.au

Background: Preterm delivery (gestation <37 completed weeks) is a major cause of neonatal mortality and morbidity. Maternal betamethasone treatment is considered an essential intervention for fetal lung maturation and neonatal survival. The placental glucocorticoid receptor (GR) is central to glucocorticoid signalling and mediating steroid effects on pathways associated with fetal growth and lung maturation but GR has not been examined in the guinea pig placenta even though this animal is regularly used as a model of preterm birth and excess glucocorticoid exposure.

Methods: Guinea pig dams received subcutaneous injections of either vehicle (175µL/kg, 0.9% sodium chloride) or betamethasone at 24 and 12 hours prior to preterm (62±1d GA) or term (68/69d GA) caesarean-section delivery. At delivery, the uterus was exposed and placentae and pups collected. Pup and organ weights were recorded. Placentae were dissected, weighed and stored at -80°C until analysis. Western blot was performed to examine GR isoform expression in nuclear and cytoplasmic extracts. GR data was analysed using non-parametric tests using SPSS.

Results: Birthweight was significantly reduced in male preterm group exposed to steroid (Bonferroni post hoc test, P=0.018). Placental weight was not significantly different between preterm or term pregnancies and was not affected by betamethasone treatment. A comparative examination of the guinea pig GR gene identified it is capable of producing seven of the eight translational GR isoforms which include GRα-A, C1, C2, C3, D1, D2, and D3. GRα-B is not produced in the Guinea Pig. Total GR antibody (Bethyl Biosciences) identified 14 specific bands from term (n=29) and 12 specific bands in preterm pregnancies (n=27). Known isoforms included GRγ (95 kDa), GRα A (94kDa), GRβ (91 kDA), GRα C (81 kDa) GRP (74kDa) GRA (65 kDa), GRα D1-3 (50-55 kDa). There were unknown proteins detected by the GR antibody including the 69, 68, 60, 48 and 38 kDa proteins. Following pre-absorption of the GR antibody with the control peptide all MW forms were removed. There were sex and gestational age differences in placenta GR isoform expression. Female placentae exposed to betamethasone had a decreased nuclear GRα A at preterm and term with no change in male placentae (KW-ANOVA, P=0.015). GRβ and GRα D3was increased in female preterm placentae exposed to betamethasone (P<0.01).

Conclusions: The current data suggests the sex specific placental response to maternal betamethasone may be dependent on the expression of a combination of GR isoforms and indicates a greater level of complexity than previously considered when only GRα A was measured and studied.

Increased prolyl hydroxylase and decreased glucocorticoid receptor are related to decreased surfactant protein in the lung of the chronically hypoxemic growth restricted sheep fetus

Sandra Orgeig[2], Erin V McGillick[1,2], Kimberley J Botting[1], Song Zhang[1], I Caroline McMillen[1] and Janna L Morrison[1]

[1]Early Origins of Adult Health Research Group; [2]Molecular & Evolutionary Physiology of the Lung Laboratory, School of Pharmacy & Medical Sciences, Sansom Institute for Health Research, University of South Australia, Adelaide, SA, Australia, 5001.
Sandra.orgeig@unisa.edu.au

Background: Experimental restriction of placental growth in the sheep results in intrauterine growth restriction (IUGR), chronic fetal hypoxemia, increased fetal cortisol concentrations and decreased lung surfactant protein (SP) expression. The mechanisms responsible for decreased SP expression are unknown.

Hypothesis: We hypothesise that decreased glucocorticoid (GC) action and/or changes in intracellular hypoxia signalling are involved in the reduced SP expression observed in the lung of the PR fetus.

Methods: Endometrial caruncles were removed from nonpregnant ewes to induce PR. Lungs were collected from Control and PR fetuses at 130-135 (n=19) and 139-145 (n=28) days gestation. qRT-PCR was used to quantify lung mRNA expression and Western blotting to quantify protein expression of molecular regulators and downstream targets of the GC and hypoxia signalling pathways. Effects of treatment (Control vs PR) and age (133 vs 140d) on the relative expression of target genes/proteins were determined using a 2-way ANOVA.

Results: Lung *SP-A*, *-B* and *-C*, but not *SP-D* mRNA expression decreased in PR fetuses at both ages. This was associated with a marked reduction in GC receptor (GR)-α and -β protein abundance and in the transcription cofactor, GATA-6. There was no upregulation of GR responsive genes (IL-1β, transforming growth factor (*TGF)-β1* and beta adrenergic receptor) in lung. Prolyl hydroxylase domain (*PHD)2* mRNA and protein and *PHD3* mRNA expression increased together with *HIF-1*α and *-1*β mRNA expression in the PR fetal lung at both ages. There was an increase in mRNA expression of several hypoxia-responsive genes in the PR fetus.

Conclusions: Changes in intracellular GC and hypoxia signalling may contribute to reduced SP expression in the lung of the PR fetus. While acute hypoxemia normally inactivates PHDs, chronic hypoxemia increased PHD abundance, which would usually prevent HIF-α signalling. This may represent a mechanism by which chronic hypoxemia contributes to the decrease in SP production in the PR fetal lung.

Comparative Effects of Endogenous and Synthetic Glucocorticoid Steroids during Mammalian Lung Development

Bennet KL Seow[1], Annie RA McDougall[1,2], Megan J Wallace[2], Anthony D Bird[1] and Timothy J Cole[1]

[1]Department of Biochemistry and Molecular Biology, Monash University, Melbourne; [2]The Ritchie Centre, MIMR-PHI Institute of Medical Research, Melbourne.
Bennet.seow@monash.edu

Background: Preterm babies are born with immature lung, which increases their risk of respiratory distress syndrome (RDS). Currently, the only treatment for lung immaturity in preterm babies is maternal administration of synthetic glucocorticoids. However there are some adverse side effects associated with synthetic glucocorticoid use, such as a decrease in overall fetal growth and delayed brain development, which are not observed with endogenous glucocorticoids. Despite their routine use, the genomic mechanisms surrounding glucocorticoid-mediated lung development remain poorly characterised.

Aims/Hypothesis: Synthetic and endogenous glucocorticoids differentially regulate specific, but different, subsets of genes leading to rapid lung maturation in the preterm, but also the inadvertent modulation of off-target genes linked to various adverse side effects.

Methods: Fetal rat lung fibroblast cells, isolated from *Sprague Dawley* rats at E20 (term ~E22), were treated for 6 hours with either synthetic (Betametasone or Dexamethasone 10^{-6}M), endogenous glucocorticoids (Corticosterone 10^{-6}M) or vehicle as a control (0.01%). After which the RNA was extracted for Next Generation RNA-sequencing (RNA-seq). Quantitative PCR was used to confirm the expression of selected gene targets.

Results: The overall gene expression profile is similar for both endogenous and synthetic glucocorticoids. However synthetic glucocorticoids modulated most of these genes to a higher degree compared to endogenous glucocorticoids. Expression of selected gene targets Cysteine-rich secretory protein LCCL domain containing 2 (*Crispld2*) and hypoxia inducible factor 3 *(Hif3a),* both of which have previously been linked to early lung development, were significantly increased ($p<0.05$) in lung fibroblast cells treated with either corticosterone, betamethasone or dexamethasone, compared to fibroblasts treated with vehicle.

Conclusion: By gaining a better understanding of the mechanisms driving glucocorticoid mediated lung development it will be possible to development better lung-specific treatments for preterm babies that retain the ability to induce rapid lung maturation, while avoiding adverse side effects.

Adrenal contribution to neurosteroidogenesis in the neonate: effect of preterm birth and progesterone replacement

Kirsten McInerney[1, 2], Hannah Palliser[1, 2], Jonathan Hirst[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: Preterm birth is a major cause of neurodevelopmental disorders. Progesterone and its metabolites allopregnanolone and DHEA have neuroprotective and developmental effects in the brain. We have previously administered progesterone to preterm guinea pig neonates to replace the functional contribution of the placenta to these neurosteroid levels. However, whilst allopregnanolone was increased, male preterm progesterone treated neonates also exhibited increased, potentially detrimental, cortisol concentrations. Adrenal contribution to neuroactive steroids after preterm birth and progesterone therapy was examined.

Aims/Hypothesis: The objectives of this study were to measure adrenal contribution to neurosteroidogenesis following preterm delivery and subsequent progesterone replacement therapy.

Methods: Guinea pig pups were delivered at either term (gestational age (GA) 69) or delivered preterm (GA 62 days). Preterm neonates were administered either vehicle or progesterone for 8 days prior to euthanasia at term equivalence. Adrenal capacity to produce allopregnanolone (5 alpha reductase type 1 and 2 [5αRI and II]), progesterone (3-β-hydroxysteroid dehydrogenase [3β-HSD]), cortisol (Cytochrome P450 11B1 [CYP11β1]) and dehydroepiandrosterone (DHEA) (Cytochrome P450 17A1 [CYP17A1]) was assessed via western blot and immunoassay.

Results: No differences in enzyme expression were found between term and preterm neonates, however plasma cortisol levels were significantly higher and DHEA significantly lower in preterm neonates than term neonates. As previously identified, cortisol concentrations were significantly increased in progesterone treated male preterm neonates compared to vehicle. This was not seen in female neonates. CYP11β1 (catalyses formation of cortisol) was lower in preterm female neonates administered progesterone compared to preterm vehicle treated female neonates.

Conclusions: Unlike male preterm neonates, preterm female guinea pig neonates appear to have the capability to regulate cortisol production by lowering the cortisol producing enzyme CYP11β1. This highlights the sex-dependant alteration in neuroactive steroid synthesis following preterm birth and subsequent progesterone replacement therapy. This work also elucidates the necessity for non – metabolisable analogues and/or sex-dependant treatments of neonates following preterm birth.

DNA methylation controls corticotropin releasing hormone (CRH) expression in human trophoblast cells

Xin Pan[2,3], Roger Smith[2,3], Rodney J Scott[2,3,4], John Fitter[2,3] & Tamas Zakar[1,2,3]

[1]Obs & Gyn, John Hunter Hospital; [2]School of Medicine & Public Health, Univ Newcastle; [3]Mothers and Babies Res Ctre & Information Based Medicine, Hunter Medical Res Institute and [4]Molecular Med, HAPS, Newcastle, NSW, Australia.
Tamas.Zakar@newcastle.edu.au

Background: Placental CRH expression increases in late pregnancy resulting in an exponential rise of maternal CRH levels towards term. The increase is predictive of gestational length suggesting that it is associated with the mechanism that determines the timing of term, preterm or post-term labour.

Aims/Hypothesis: We have explored the possibility that the gestational programming of CRH production by the placenta is controlled by the methylation of the *CRH* gene promoter in the trophoblast cells.

Methods: Cytotrophoblasts from term placentas (n=3) were maintained in primary culture for 72 h with or without stimulation with 8-Br-cAMP. *CRH* mRNA and peptide levels were determined with qRT-PCR and RIA, respectively. Methylation of the 9 CpG sites in the *CRH* proximal promoter was determined by bisulfite sequencing. Promoter binding of pol-II, TBP and phospho-(p)CREB was measured by chromatin immunoprecipitation. The methylation of the transcription factor-bound, chromatin immunoprecipitated promoter DNA was determined by bisulfite sequencing.

Results: *CRH* mRNA level increased 60-fold by 72 h and was further stimulated by 8-Br-cAMP. CRH peptide output had similar kinetics. All CpG dinucleotides were partially methylated (in 17-70% of the alleles) including CpG-2 located in the cAMP-response element (CRE), which is inactive when modified by methylation. Methylation frequency was unaltered during culture and by 8-Br-cAMP (Fisher's exact). *CRH*-promoter binding of pol-II increased ~10-fold by 8-Br-cAMP. Pol-II-bound promoter DNA had a characteristic high CpG methylation pattern. Phospho-CREB bound to the CRE region increasingly in response to 8-BrcAMP, but to a different population of *CRH* alleles with a distinct methylation pattern. TBP binding increased during syncytialisation, but not in response to 8-Br-cAMP. TBP-bound alleles were enriched in methylation patterns recognizing pol-II or pCREB.

Conclusions: Methylation pattern determines the number of pol-II-bound transcriptionally active *CRH* alleles in the trophoblast population. The pCREB-CRE interaction is not involved in *CRH* gene regulation in native trophoblast chromatin. We propose that adverse conditions that abrogate DNA methylation program placental CRH expression epigenetically by altering the number of transcriptionally active *CRH* gene copies in the trophoblast population.

Session 7

Session 7: Chairs – Jane Pillow and Keiji Suzuki			
2:00	**A38**	Erin McGillick (L)	Maternal antioxidant treatment increases expression of genes regulating hypoxia signalling, sodium movement & surfactant maturation in the fetal lung
2:15	**A39**	Ishmael Inocencio (E)	Ultra-high dose nanoparticles increased markers of lung injury and inflammation in the preterm lamb
2:30	**A40**	Paris Papagianis (E)	Optimising continuous positive airway pressure for preterm infants
2:45	**A41**	Lauren Kerr (ECR)	Sustained inflations impact lung aeration at birth
3:00	**A42**	Domenic LaRosa (ECR)	Determining the optimal dose of EPO to reduce ventilator induced lung injury
3:15	**General discussion**		

Maternal antioxidant treatment increases expression of genes regulating hypoxia signalling, sodium movement & surfactant maturation in the fetal lung

Erin V McGillick[1], Sandra Orgeig[1], Beth J Allison[2], Kirsty L Brain[2], Youguo Niu[2], Nozomi Itani[2], Katie L Skeffington[2], Andrew D Kane[2], Emilio A Herrera[2], Janna L Morrison[1] & Dino A Giussani[2]

[1]Sansom Institute for Health Research, University of South Australia, Adelaide, Australia; [2]Department of Physiology, Development & Neuroscience, University of Cambridge, UK.
erin.mcgillick@mymail.unisa.edu.au

Background: In the fetus, the balance of pro- and anti-oxidants is essential to negate detrimental effects of oxidative stress and promote lung maturation. Antioxidant molecules improve respiratory function (Britton et al. *Am J Resp Crit Care* 151:1383, 1995), however, in the fetal lung the outcome and biological mechanism of action are unknown.

Aim: To determine the effect of maternal antioxidant treatment on expression of genes regulating processes vital to the successful fetal transition to air-breathing.

Methods: Chronically catheterised pregnant sheep carrying male singletons received saline (S; n=8) or Vitamin C (VC; maternal 200mg/kg i.v. daily, n=9) from 105-138d gestation (term, ~145d). At 138d gestation, fetuses were delivered, weighed and tissues collected. qRT-PCR was used to quantify mRNA expression of genes regulating hypoxia signalling (*PHD-1*, *-2*, *-3*, *HIF-1α*, *-2α*, *-3α*, *-1β*, *VEGF*, *JMJD1A*, *ADM* & *GLUT1*), lung liquid reabsorption (sodium movement (*ENAC-α*, *-β* & *-γ*, *ATPase-α1* & *-β1* subunits) and water transport (*AQP-1*, *-3*, *-4* & *-5*)) and surfactant maturation (*SP-A*, *-B*, *-C*, *-D*, *PCYT1A* & *ABCA3*) in the fetal lung. Data were analysed by the Student's unpaired *t*-test. $P<0.05$ was considered significant.

Results: Fetal body and relative lung weight were similar between groups. Fetuses exposed to maternal VC showed increased lung mRNA expression of *PHD-3*, *HIF-2α*, *-3α*, *ADM*, *ENAC-α*, *-β*, *ATP1-α1*, *-β1*, *SP-B* and *ABCA3.*

Conclusions: Maternal antioxidant treatment increases expression of genes regulating sodium movement and surfactant development in the fetal lung. These maturational effects are associated with an increased expression of genes regulating hypoxia signalling.

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Ultra-high dose nanoparticles increased markers of lung injury and inflammation in the preterm lamb

Inocencio IM[1], Plebanski M[2], Xiang S[2], Tolcos M[1], Zahra V[1], Nguyen V[1], Lim T[1], LaRosa D[1], Moss TJ[1], Polglase GR[1]

[1]The Ritchie Centre, MIMR-PHI Institute of Medical Research and Department of Obstetrics and Gynaecology, Monash University, Melbourne, Australia; [2]Department of Immunology, Central Clinical School, Faculty of Medicine, Nursing and Health Sciences, Alfred Hospital Campus, Monash University, Melbourne, Australia.
ishmael.inocencio@monash.com.au

Background: Preterm infants often possess an immature and underdeveloped respiratory system. To survive the transition from *intra* to *extra* uterine life respiratory support in the form of mechanical ventilation is often required. The initiation of mechanical ventilation (MV) can cause lung inflammation and injury with potential life-long adverse respiratory, cardiac and neurological sequelae. Lung inflammation can be reduced by inert 50nm polystyrene nanoparticles, shown by their ability to reduce allergic inflammation in the lungs of adult mice. However, use of nanoparticles as therapeutics is contentious due to possible toxicity.

Aims/Hypothesis: To investigate the efficacy of prophylactically administered nanoparticles to reduce ventilation induced lung injury (VILI) in preterm lambs.

Methods: Preterm lambs (0.83 gestation) were delivered by caesarean section and either immediately euthanized (n=5) or injuriously ventilated for 15 min either with (n=6) or without (n=5) prophylactic nanoparticles (3% in 2 ml saline). Ventilation was subsequently continued for a total of 2 h to allow for manifestation of VILI. Lung tissue was frozen and collected for qRT-PCR analysis of proinflammatory cytokines and early injury gene marker expression. The right upper lung lobe was collected, sectioned and stained with haematoxylin and eosin for assessment of lung injury by assessing; tissue morphology, haemorrhage, immune cell infiltration and epithelial sloughing.

Results: Injurious ventilation increased all markers of inflammation and injury compared to unventilated controls (UVC). However, nanoparticle administration further increased proinflammatory cytokines (IL-1β, IL-6 and IL-8) and early lung injury genes (CTGF, ERG1 and CYR61) compared to lambs without nanoparticles. Injurious ventilation also significantly increased histological markers of lung injury compared to UVC. Nanoparticle administration further increased lung wall thickness, immune cell infiltration and epithelial sloughing.

Conclusions: Prophylactic nanoparticle administration increased lung inflammation and injury, indicating at this dose inert 50 nm polystyrene nanoparticles are detrimental to the lung. Further dosage studies are required to examine potential efficacy for lung protection.

Optimising continuous positive airway pressure for preterm infants

Papagianis PC[1,2], Nguyen V[1], Polglase GR[1], Moss TJ[1,2]

[1]The Ritchie Centre, MIMR-PHI Institute of Medical Research, Clayton, Melbourne, Australia; [2]Department of Obstetrics and Gynaecology, Monash University, Clayton, Melbourne, Australia.
paris.papagianis@monash.edu

Background: Preterm birth is defined as birth of an infant before 37 completed weeks of gestation, occurring in 8% of live births in Australia. Birth before term results in organ immaturity and, due to poor respiratory development, and hence function, infants often require respiratory support. However, all current techniques of ventilator support, while they are life saving, injure the preterm lugs. Continuous positive airway pressure (CPAP) is commonly used in Australian neonatal intensive care units (NICUs) and is considered non-invasive as it can be delivered via nasal prong(s), without requiring intubation. CPAP is associated with overall less lung injury and long-term complications when compared to intubating during mechanical ventilation. However, despite its advantages, established use of CPAP in the clinic has occurred without thorough knowledge as to how the device interacts with the neonate to improve respiratory outcomes. CPAP is only applied to infants who are making some spontaneous breathing efforts but cannot maintain respiration independently. Devices used to generate CPAP include ventilator-derived CPAP (V-CPAP) and bubble CPAP (B-BPAP), where pressure is delivered via a bubbling water seal. A third CPAP modality, high amplitude B-CPAP (HAB-CPAP), shows potential in enhancing respiratory support experimentally, more-so than V- and B- CPAP, but is yet to be used in the clinic.

Hypothesis: Provision of breathing support using bubbling CPAP modalities will transmit pressure variations to the distal lung, thus reducing respiratory effort and enhancing oxygenation levels and pulmonary haemodynamics compared to V-CPAP.

Methods: Instrumentation via aseptic surgery was performed in fetal sheep at 130 days gestation (n=8; term ~147 days). After 3 days of recovery, lambs were delivered via caesarean section and received mechanical ventilation until stabilized, as defined by regular spontaneous breathing efforts. Following stabilization, lambs were weaned onto CPAP, receiving V-CPAP followed by either B- or HAB- CPAP for 15-minute intervals. Choice of succeeding V-CPAP with B- or HAB- CPAP was randomised. Pressure was set at 8 cmH_2O for all CPAP modalities. Measurements at study completion included arterial blood gases, arterial pressure, pulmonary pressure, inspiratory pressure, pulmonary blood flow, tidal volume, airway pressure and respiratory rate.

Results/Conclusions: Many lambs did not survive until the day of experimentation, resulting in small animal numbers for this study. In animals that successfully underwent CPAP no profound physiological differences between the three CPAP modalities were observed.

Sustained inflations impact lung aeration at birth

Lauren Kerr[1] Genevieve Buckley[2] Megan Wallace[1,3] Marcus Kitchen[2] Melissa Siew[1] Helena Cangadis-Douglass[1] Charles Roehr[1,4] and Stuart Hooper[1,3]

[1]The Ritchie Centre, MIMR-PHI Institute of Medical Research, VIC 3168, Australia; [2]Dept of Physics, Monash University, VIC 3800, Australia; [3]Dept of Obstetrics and Gynaecology Monash University, VIC 3168, Australia, [4]John Radcliffe Hospital, Oxford University, OX3 9DU, UK.
Lauren.Kerr@monash.edu

Background: In preterm infants, the cardio-pulmonary transition at birth can be impaired, leading to compromised respiratory function and the requirement for respiratory support. Recent studies have shown that a sustained inflation (SI) is an effective method for aerating the lungs at birth.

Method: To ascertain whether the rate and volume of a SI affects the distribution of lung aeration, we examined the effect of different SI strategies in newborn rabbits. We analysed propagation-based phase contrast (PBPC) X-ray images of rabbit lungs inflated at birth.

Results: We observed that soon after the onset of the SI, the lung within ribs 1-3 (upper region) inflates quickly and the volume remains constant whilst the lateral lung region continues to expand and lower lung regions inflate. The lung volume within ribs 4-5 (middle region) inflates and reaches maximum volume after the upper region. Ribs 6-8 (lower region) behaves as a reservoir for excess lung gas volume, expanding to accommodate large volumes of air and reaching its maximal volume well after the upper and middle regions of the lung. Lung overextension or bulging was observed in the upper and middle lung regions in some pups with large SI volumes (40 mL/kg), significantly less lung bulging was seen with moderate SI volumes (20 mL/kg [upper region p=0.006, middle region p=0.015]). No significant change in lung bulging was evident with slow inflations compared to rapid inflations.

Conclusion: Data indicates that sustained inflations uniformly aerate the upper lung regions before the lower regions and that lung overextension is due to large tidal volumes.

Determining the optimal dose of EPO to reduce ventilator induced lung injury

Domenic LaRosa[1], Tim Moss[1], Valerie Zahra[1], Mary Tolcos[1], Andy Gill[2], Martin Kluckow [3], Laura Bennet[4], Mikee Inocencio[1], Graeme Polglase[1]

[1]The Ritchie Centre, MIMR-PHI Institute of Medical Research & Department of Obstetrics and Gynaecology, Monash University, Melbourne, Australia; [2]Department of Neonatology, Royal North Shore Hospital and University of Sydney, Sydney; [3]Centre for Neonatal Research and Education, The University of Western Australia, Australia. [4]Department of Physiology, The University of Auckland, Auckland, New Zealand.
graeme.polglase@monash.edu

Background: Erythropoietin (EPO) is being trialled in preterm infants to reduce preterm brain injury. We previously demonstrated that early EPO administration (5,000IU) to ventilated preterm lambs resulted in increased ventilation induced lung injury (VILI)[1]. We aimed to determine whether early administration of lower doses of EPO could protect the preterm lung form VILI.

Method: Ventilation was initiated in preterm lambs (125 ± 1 (SD) days gestation) targeting 12-15 ml/kg for the first 15 minutes. Lambs were subsequently ventilated with 7 ml/kg and 5 cmH_2O PEEP for a total of 2 h. Lambs were randomised to either control (n=5), or 300 IU (n=5) 1000 IU (n=5) or 3000 IU (n=5) EPO. Lung tissue was snap frozen and collected for qRT-PCR analysis of proinflammatory cytokines and early lung injury gene marker expression and compared to unventilated controls (UVC).

Results: The initiation of ventilation resulted in significant increases in interleukin (IL)-1β, IL-6 and IL-8 and early lung injury genes CTGF, ERG1 and CYR61 compared to UVC. All doses of EPO did not significantly increase markers of inflammation and injury compared to ventilated controls, with 3000 IU having an apparent reduction (non-significant) compared to the other doses.

Conclusions: Early administration of EPO to ventilated preterm lambs at low doses does not exacerbate ventilation induced lung inflammation and injury.

[1] Polglase et al. J Physiol. 2014 May 1;592(Pt 9):1993-2002.

Session 8

Session 8: Chairs – Laura Bennet and Tamás Zakár			
4:00	**A43**	Emily Cohen (E)	Size at birth and gender influence cerebral oxygenation in preterm neonates during the first three days of life
4:15	**A44**	Kathryn Martinello (M)	Gestational age related alterations in interleukin-6 *trans*-signalling may predispose to bronchopulmonary dysplasia
4:30	**A45**	Nadia Bellofiore (E)	Pseudopregnancy induction in the spiny mouse (*Acomys cahirinus*)
4:45	***A46**	Tamara Yawno	Progesterone treatment of the growth restricted fetus - a natural therapy
4:53	***A47**	Sogand Hosseini (M)	Maternal stress in pregnancy reduced mRNA expression of placental growth related factors
5:01	***A48**	Danielle Burgess (E)	Does periconceptional ethanol exposure in the rat offspring alter the hypothalamic-pituitary-adrenal axis and subsequent stress responses?
5:09	***A49**	Courtney McDonald (ECR)	How does neuroinflammation drive brain injury following hypoxia-ischemia?
5:17	**General discussion**		

Size at birth and gender influence cerebral oxygenation in preterm neonates during the first three days of life

Emily Cohen[1,2], Willem Baerts[1], Thomas Alderliesten[1], Jan B Derks[3], Petra Lemmers[1], Frank van Bel[1]

[1]Department of Neonatology, Wilhelmina Children's Hospital/Utrecht University Medical Center, Utrecht, The Netherlands; [2]The Ritchie Centre, MIMR-PHI Institute and Department of Paediatrics, Monash University, Melbourne, Victoria, Australia; [3]Department of Obstetrics, Wilhelmina Children's Hospital /Utrecht University Medical Centre, Utrecht, The Netherlands.
Emily.cohen@monash.edu

Background: The small-for-gestational age (SGA) fetus redistributes its cardiac output to maximise oxygen and nutrient supply to the brain ("brain-sparing"). Currently little is known about the postnatal course of brain-sparing. As neuroprotection forms a significant part of care for the preterm infant, it is important to explore this topic.

Aims/Hypothesis: To test the hypothesis that SGA preterm neonates have increased cerebral blood flow as indicated by increased cerebral oxygenation during the early neonatal period compared to appropriate-for-gestational age (AGA) preterm neonates.

Methods: Regional cerebral oxygen saturation (rScO2) and cerebral fractional tissue oxygen extraction (cFTOE) recordings obtained by near-infrared spectroscopy (Invos 5100c and SAF-MB, Covidien, Mansfield, MA, USA) throughout the first 72 hours of life were compared between 90 (57 males) SGA (birth weight <10th percentile) and 180 (114 males) AGA (birth weight 20th – 80th percentile) neonates, matched for gender, GA, blood pressure and respiratory support.

Results: SGA males had significantly higher rScO2 and lower cFTOE than all other groups ($p < 0.01$). AGA males and SGA females showed comparable rScO2 ($p = 0.72$) and cFTOE ($p = 0.49$). AGA females had lower rScO2 and higher cFTOE than the other three groups ($p < 0.01$). There was an independent effect of both gender and size at birth, in which males exhibited higher rScO2 and lower cFTOE than females ($p < 0.01$) and SGA neonates demonstrated higher rScO2 and lower cFTOE than AGA neonates ($p<0.01$). This independent effect may explain the overlap seen between AGA males and SGA females.

Conclusions: Both size at birth and gender influence cerebral oxygenation and oxygen extraction throughout the first three days of life. Further longitudinal studies, in which gender is taken into account, are required to examine the clinical consequences of these findings.

Gestational age related alterations in interleukin-6 *trans*-signalling may predispose to bronchopulmonary dysplasia

Martinello K[1,2], Stark MJ[1,2], Crawford T[1], Andersen CC[1,2], Hodyl NA[1,2]

[1]Department of Neonatal Medicine, Women's and Children's Hospital, Adelaide, South Australia; [2]The Robinson Research Institute, School of Paediatrics and Reproductive Health, University of Adelaide, Adelaide, South Australia.
kathryn.martinello@health.sa.gov.au

Background: Interleukin-6 trans-signalling occurs following binding of IL-6 to the soluble IL-6 receptor (sIL-6R) and is pro-inflammatory. Binding of soluble gp130 (sgp130) to sIL-6R inhibits this process. With IL-6 implicated in the pathogenesis of bronchopulmonary dysplasia (BPD), we characterised the influence of gestational and postnatal age on this system and investigated its relationship to the development of BPD in very preterm neonates.

Methods: Plasma was collected from infants <30 weeks gestation (control group n=27, BPD group n=13) on day 1-2, 7, 14, and 28 of life. BPD was diagnosed by a physiologic challenge at 36 corrected weeks gestation. IL-6, sIL-6R and sgp130 were measured by ELISA.

Results: Gestational age was inversely correlated with day1-2 IL-6 ($p=0.02$) and positively correlated with day 1-2 sgp130 ($p<0.01$). While IL-6, sIL-6R and sgp130 did not change with postnatal age; at each study time-point sgp130 and sIL-6R were positively correlated ($p<0.001$). The ratio of sgp130:sIL-6R, representing anti-trans-signalling, was lower on day 1-2 in infants who developed BPD ($p=0.04$) and IL-6 higher in the BPD group by day 28 ($p=0.06$).

Conclusion: The positive relationship between sgp130 and sIL-6R seen across the neonatal period suggests a dynamic homeostatic regulatory mechanism. However, the inverse relationship between gestational age and sgp130 suggests the most preterm infants have limited capacity to regulate IL-6 trans-signalling.

The reduced sgp130:sIL-6R ratio on day 1-2 of life in those who subsequently develop BPD suggests that early dysregulation of the IL-6 trans-signalling pathway may contribute to the pathogenesis of this significant neonatal morbidity.

Pseudopregnancy induction in the spiny mouse (*Acomys cahirinus*)

Nadia Bellofiore[1], Tania Bernadette Fernandez[2], Jared Mamrot[1], David W Walker[1], Peter Temple-Smith[2], Hayley Dickinson[1]

[1]The Ritchie Centre, MIMR-PHI Institute of Medical Research, Clayton, Victoria, Australia; [2]Obstetrics and Gynaecology, Monash University, Clayton, Australia.
nbel10@student.monash.edu

Background: The spiny mouse is a precocial rodent, with hormonal and fetal developmental similarities to human pregnancy. Having extensively studied pregnancy and fetal development in this species, we now propose to undertake comprehensive embryology based studies. To do so, we need a robust technique to induce pseudopregnancy.

Aims/Hypothesis: We sought to trial 3 protocols previously used in rodents in the spiny mouse. We hypothesised that progesterone would induce uterine decidualisation and establish pseudopregnancy.

Methods: Female spiny mice aged between 90-150 days were divided randomly into one of several groups (Table 1). Spiny mice were deemed pseudopregnant if presenting with an extended luteal phase, characterised by >4 consecutive days of Type 5 leukocytic smears.

Table 1: Treatment groups and vaginal smear protocols for inducing pseudopregnancy

Group #	Subjects (n)	Treatment	Smears Conducted
1	10	Control	Every two days
2	5	Progesterone 4mg	Daily after treatment
3	5	Progesterone 4mg	Day 3 onwards after treatment
4	5	Mechanical Stimulation	Daily
5	5	Mechanical Stimulation	Day 3
6	5	Sterile Mating	Daily
7	10	Sterile Mating	Day 3
8	5	Progesterone 2 mg	Day 3
9	5	Progesterone 5 mg	Day 3

Results: The average length of luteal phase in untreated animals was 2.8 ± 0.2 days. This was significantly prolonged by 3-5 days in most groups, excluding 2 and 5. Though the luteal phase was significantly prolonged in 7, 50% of subjects exhibited delayed pseudopregnancy. 40% of subjects from 6 experienced prolonged oestrus by 1-2 days.

Conclusion: We found a single dose of progesterone (2-5 mg) was the most efficacious method of immediate pseudopregnancy induction in spiny mice. Altered concentrations did not have an effect on luteal phase length, hence any dosage within this range may be used. Ongoing analysis will confirm decidualisation and hormone profiles. Embryo transfers will be conducted to confirm protocol success.

Maternal stress in pregnancy reduced mRNA expression of placental growth related factors

Sogand S. Hosseini[1], James SM Cuffe[2], Andrew J Jefferies[1], Karen M. Moritz[2], Mary E. Wlodek[1]

[1]Department of Physiology, The University of Melbourne, Parkville, Victoria, Australia; [2]School of Biomedical Sciences, University of Queensland, St. Lucia, Queensland, Australia.
Sogandh@students.unimelb.edu.au

Background: Intrauterine growth restriction increases the risk of adult diseases that can be transmitted to the next generation (F2). F1 mothers born small and exposed to stressors during late gestation can adversely impact on fetal and placental growth and development.

Aims/Hypothesis: To determine if maternal stress during pregnancy in F1 growth restricted female rats alters placental gene expression and fetal and placental growth.

Methods: Uteroplacental insufficiency was induced by bilateral uterine vessel ligation (Restricted) or sham surgery (Control) on day 18 of pregnancy of (F0) females. F1 Control and Restricted female offspring were mated with normal males and allocated to Unstressed or Stressed groups. Physiological stressors (24h metabolic cage, tail cuff blood pressure, glucose tolerance test) were introduced during late pregnancy in the stressed group. F2 fetal body and placental weights were measured at embryonic day 20 and placentae were collected for gene expression analysis.

Results: F2 offspring from F1 mothers exposed to maternal stress had significantly reduced fetal weight, placental weight and fetal placental ratio ($p<0.05$). Labyrinth zone gene expression in both F2 male and female offspring from F1 stressed mothers was reduced for placental mineralocorticoid receptor (*Nr3c2*), Corticotropin-releasing hormone receptor 1 (*Crhr1*), hydroxysteroid11-beta dehydrogenase 2 (*Hsd11b2*) and system A amino acid transporter 2 (*Slc38a2*); However, there were no significant differences between Control and Restricted groups.

When the effects of stress on placental outcomes were determined in restricted dams, gene expression for the glucocorticoid receptor (Nr3c1) in F2 stressed, restricted male fetuses were significantly reduced ($P<0.05$). Glucose transporters 1 and 3 (Slc2a1, Slc2a3) and insulin-like growth factor 2 (Igf2) were all reduced following maternal stress in placentae from males of both the Control and Restricted groups. In placentae from F2 females, mRNA levels were reduced following maternal stress in the Control group but not the Restricted group suggesting adaptations in females not present in males

Conclusions: Exposure to maternal stress gave rise to F2 offspring low birth weight. Maternal stress induced alterations to the expression of genes related to placental stress, nutrient transport and placental growth. These adaptations may impair fetal development and program disease in offspring.

Does periconceptional ethanol exposure in the rat offspring alter the hypothalamic-pituitary-adrenal axis and subsequent stress responses?

Danielle J Burgess, James S M Cuffe & Karen Moritz

School of Biomedical Sciences, The University of Queensland, Brisbane, QLD, Australia.
d.burgess@uq.edu.au

Background: Maternal ethanol (EtOH) consumption throughout gestation can impair fetal development and program lifelong physiological outcomes. Less is known about alcohol consumption around the time of conception. This is of significant importance as 47.3% of women consume alcohol in the first three weeks of pregnancy, a period known as the periconceptional (PC) period. The hypothalamic pituitary adrenal (HPA) axis is highly susceptible to chronic maternal alcohol consumption, with previous studies revealing alterations in stress responses and behaviour. It is therefore important to determine the impact of PCEtOH exposure on HPA function.

Aims and hypotheses: It is hypothesised that maternal PCEtOH exposure will increase HPA function in the mother, placenta and offspring. The aim of this study is to investigate the effects of maternal PCEtOH exposure on the expression of genes and proteins related to HPA function in the maternal adrenal gland, the placenta and offspring adrenal gland and to establish corticosterone levels in the mother, fetus and offspring.

Methods: Sprague-Dawley rats were exposed to a liquid diet ± 12% v/v ethanol (n=12 per group) during the periconceptional period from 4 days prior to pregnancy (E-4) to embryonic day 4 (E4). Dams were killed at E20 for the collection of plasma, maternal adrenals, placentas and fetal adrenals or allowed to litter down for offspring studies. Plasma and adrenal glands has been collected from 12 month offspring. RNA and protein will be extracted for QPCR and western blot analysis respectively. A RIA will be used to measure corticosterone in plasma at all ages collected.

Preliminary and Expected Results: Adrenal weight at E20, 8 months and 12 months was not significantly different in animals exposed to PCEtOH compared to control. In addition, preliminary studies found no significant change in adrenal mRNA expression of Cyp11a1 and Cyp21a1 at 12 months of age. However, as the adrenal stress pathway is cyclic, plasma corticosterone levels may still be altered. It is expected that there will be PCEtOH induced alterations in maternal plasma corticosterone and expression of enzymes within the adrenal steroidogenesis pathway. Subsequent changes in key HPA factors within the placenta are also anticipated.

Conclusions: Preliminary data is yet to observe any changes in regards to PCEtOH on adrenal steroidogenesis in aged offspring. However, research will continue to investigate alternations in both basal and regulatory stress pathways throughout gestation.

Progesterone treatment of the growth restricted fetus - a natural therapy

Tamara Yawno[1], David W Walker[1,2], Jan Loose[1], Graham Jenkin[1,2] and Suzanne L Miller[1,2]

[1]The Ritchie Centre, MIMR-PHI; [2]Department of Obstetrics and Gynaecology, Monash University, Clayton, Australia.
tamara.yawno@monash.edu

Background: IUGR fetuses are likely to be born preterm, and therefore the mother usually receives glucocorticoids such as betamethasone to promote fetal lung maturation. We have however shown that antenatal betamethasone significantly decreases the essential neurosteroid allopregnanolone (AP) in the fetal brain and causes cellular injury (Yawno et al, 2014).

Aims/Hypothesis: In this study of IUGR fetal sheep, we examined if exogenous progesterone - the natural substrate for AP synthesis - is able to maintain fetal brain AP and reduce brain injury following maternal betamethasone treatment.

Methods: Single umbilical artery ligation (SUAL) was performed to induce IUGR at 103 days gestation in fetal sheep. Ewes were given betamethasone (or saline) on days 10 and 11 after surgery, and progesterone (or vehicle) on days 9, 10 and 11. After euthanasia on day 124, fetal brains were collected to determine AP and progesterone levels and brain histopathology.

Results: In IUGR fetuses, betamethasone treatment reduced fetal brain AP concentration (1.3 ± 0.6 pmol/ml) compared to control+saline animals (5.1 ±1.2 pmol/ml; $p<0.05$), while progesterone treatment prevented this decrease (5.2 ± 0.6 pmol/ml). Whereas betamethasone treatment increased the number of iba-1+ inflammatory cells in fetal brain white matter (685 ±180 vs [saline control] 279 ± 98 cells/mm2), this increase was significantly reduced in the progesterone+BM group (300 ± 122 cells/mm2). Progesterone treatment also reduced the number of apoptotic cells (caspase-3+) and improved myelination (CNPase+ staining) compared to non-progesterone treated animals.

Conclusions: This study confirms that some of the deleterious effects of maternally-administered glucocorticoids on the developing brain arise because of suppression of endogenous AP synthesis. We show that co-treatment with progesterone prevents the loss of this essential neurosteroid, and ameliorates brain injury following betamethasone in IUGR fetuses. Progesterone may be an effective adjuvant neuroprotective therapy that can be used clinically when preterm birth is likely to occur.

How does neuroinflammation drive brain injury following hypoxia-ischemia?

Courtney McDonald, Graham Jenkin and Suzie Miller.

The Ritchie Centre, MIMR-PHI Institute of Medical Research, Clayton, VIC 3168, Australia.
courtney.mcdonald@monash.edu

Background: Cerebral Palsy is the most common cause of physical disability in childhood and there is no cure. We know that episodes of acute hypoxia can contribute to injury, involving mechanisms including oxidative stress, excitotoxicity, apoptosis and inflammation. However, the neuroinflammatory response is very important to injury as it can propagate inflammation, and can increase oxidative stress and apoptosis. Most of the research into perinatal brain inflammation has focused on the role of microglia and macrophages, but in adult neuroinflammatory diseases, like multiple sclerosis, breakdown of the blood brain barrier causes entry of pathogenic T cells (Th1 and Th17 cells) into the CNS and orchestrates the infiltration of additional immune cells, like cytotoxic T-cells, B-cells, dendritic cells and macrophages. If these cells are left unchecked they can cause myelin breakdown and axonal damage, which are characteristic neuropathologies in perinatal brain injury. The role that each of these immune cell types play in perinatal brain injury is not known.

Aims/Hypothesis: We hypothesise that Th1 and Th17 cells are involved in the development and progression of the neuroinflammatory response that drives perinatal brain injury. We aim to characterise the role of these cells following a neonatal hypoxic-ischemic (HI) insult.

Methods: Using the Rice-Vannucci rodent model of perinatal HI, on postnatal day 5, mice pups will undergo surgery to occlude the left carotid artery, and will be placed in a hypoxic chamber (10% oxygen) for 60 minutes. Post HI injury, five time points will be analysed - 12 hours, 24 hours, 2 days, 3 days and 7 days. At each of these time points the brain and spleen will be collected and immune cells will be isolated by enzymatic digestion and phenotyped using flow cytometry. We will quantify T-cells (including subsets), B-cells, dendritic cells, and macrophages/microglia (M1 and M2). We will also collect brains to perform immunohistochemistry and stain for each of the cell types mentioned to ascertain the areas of the brain they are infiltrating and how this relates to injury.

Conclusions: We will characterise the cells and mechanisms that contribute to the neuroinflammatory response and their role in perinatal brain injury. We will then be able to develop novel therapies that can target those pathways and reduce the incidence and severity of cerebral palsy.

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Registrants

Dr. Lisa Akison The University of Queensland Brisbane, QLD l.akison@uq.edu.au	**Dr. Ali Al-Rubaie** Monash University Melbourne, VIC ali.hani@monash.edu	**Ms. Lia Amanda** MIMR-PHI Institute of Medical Research Melbourne, VIC lalia1@student.monash.edu
Ms. Kristina Anevska The University of Melbourne Melbourne, VIC **k.anevska@latrobe.edu.au**	**Ms. Jade Barbuto** MIMR-PHI Institute of Medical Research Melbourne, VIC jade.barbuto@monash.edu	**Ms. Sam Barton** MIMR-PHI Institute of Medical Research Melbourne, VIC samantha.barton@monash.edu
Miss. Nadia Bellofiore MIMR-PHI Institute of Medical Research 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. Corinna Binder MIMR-PHI Institute of Medical Research Melbourne, VIC corinna.binder@monash.edu	**Dr. Robert Bischof** MIMR-PHI Institute of Medical Research Melbourne, VIC rob.bischof@monash.edu	**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	**Ms. Sheena Bouch** Monash University Melbourne, VIC sheena.bouch@monash.edu	**Dr. Chloe Bourguignon** MIMR-PHI Institute of Medical Research Melbourne, VIC chloe.bourguignon@monash.edu
Ms. Jessica Briffa The University of Melbourne Melbourne, VIC **jgriffith@student.unimelb.edu.au**	**Miss. Danielle Burgess** The University of Queensland Brisbane, QLD d.burgess@uq.edu.au	**Miss. Lara Bush** MIMR-PHI Institute of Medical Research Melbourne, VIC lebus2@student.monash.edu
Dr. Hazel Carlisle Canberra Hospital Canberra, ACT Hazel.Carlisle@act.gov.au	**Dr. Margie Castillo-Melendez** MIMR-PHI Institute of Medical Research Melbourne, VIC margie.castillo-melendez@monash.edu	**Ms. Jean Cheong** The University of Melbourne Melbourne, VIC jncheong@student.unimelb.edu.au
Mrs. Sabiha Chowdhury University of New South Wales Sydney, NSW sabiha.chowdhury@student.unsw.edu.au	**Prof. Vicki Clifton** Translational Research Institute Brisbane, QLD vicki.clifton@adelaide.edu.au	**Dr. Emily Cohen** MIMR-PHI Institute of Medical Research Melbourne, VIC emily.cohen@monash.edu
Prof. Jeffrey Craig The Royal Children's Hospital Melbourne, VIC jeff.craig@mcri.edu.au	**Miss. Tara Crawford** The University of Adelaide Adelaide, SA tara.crawford@student.adelaide.edu.au	**Dr. Kelly Crossley** MIMR-PHI Institute of Medical Research Melbourne, VIC kelly.crossley@monash.edu

Dr. James Cuffe The University of Queensland Brisbane, QLD j.cuffe1@uq.edu.au	**Mr. Jack Darby** The University of Adelaide Adelaide, SA jack.darby@mymail.unisa.edu.au	**Dr. Robert De Matteo** Monash University Melbourne, VIC robert.dematteo@monash.edu
Miss. Sarah Delforce University of Newcastle Newcastle, NSW c3145271@uon.edu.au	**Dr. Hayley Dickinson** MIMR-PHI Institute of Medical Research Melbourne, VIC hayley.dickinson@monash.edu	**Ms. Melinda Dolan** Monash University Melbourne, VIC melinda.dolan@monash.edu
Ms. Emily Dorey The University of Queensland Brisbane, QLD e.dorey@uq.edu.au	**Ms. Rebecca Dyson** University of Newcastle Newcastle, NSW rebecca.dyson@uon.edu.au	**Dr. Yvonne Eiby** The University of Queensland Brisbane, QLD y.eiby@uq.edu.au
Dr. Stacey Ellery MIMR-PHI Institute of Medical Research Melbourne, VIC stacey.ellery@monash.edu	**Dr. Robert Galinsky** The University of Auckland Auckland, New Zealand r.galinsky@auckland.ac.nz	**Dr. Kathy Gatford** The University of Adelaide Adelaide, SA kathy.gatford@adelaide.edu.au
Ms. Madeline Goss MIMR-PHI Institute of Medical Research Melbourne, VIC mgos6@student.monash.edu	**Ms. Jessica Gugusheff** The University of Adelaide Adelaide, SA jessica.gugusheff@adelaide.edu.au	**Prof. Richard Harding** Monash University Melbourne, VIC richard.harding@monash.edu
Mrs. Nadia Hale MIMR-PHI Institute of Medical Research Melbourne, VIC nadia.hale@monash.edu	**Ms Olivia Henry** MIMR-PHI Institute of Medical Research Melbourne, VIC ojhen2@student.monash.edu	**Miss. Erandi Hewawasam** The University of Adelaide Adelaide, SA erandi.hewawasam@adelaide.edu.au
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** MIMR-PHI Institute of Medical Research Melbourne, VIC stuart.hooper@monash.edu
Prof. Rosemary Horne MIMR-PHI Institute of Medical Research Melbourne, VIC rosemary.Horne@monash.edu	**Ms. Sogand Hosseini** The University of Melbourne Melbourne, VIC sogandh@student.unimelb.edu.au	**Mr. Mikee Inocencio** MIMR-PHI Institute of Medical Research Melbourne, VIC imino1@student.monash.edu
Dr. Demelza Ireland The University of Western Australia Perth, WA demelza.ireland@uwa.edu.au	**Ms. Jacinta KalischSmith** The University of Queensland Brisbane, QLD jacinta.kalischsmith@uqconnect.edu.au	**Dr. Lauren Kerr** MIMR-PHI Institute of Medical Research Melbourne, VIC lauren.kerr@monash.edu
Ms. Delphi Kondos-Devcic MIMR-PHI Institute of Medical Research Melbourne, VIC dekon1@student.monash.edu	**Dr. Anil Lakkundi** Hunter Medical Research Institute Newcastle, NSW anil.Lakkundi@hnehealth.nsw.gov.au	**Mr. Justin Lang** MIMR-PHI Institute of Medical Research Melbourne, VIC justin.Lang@monash.edu

Mr. Domenic LaRosa MIMR-PHI Institute of Medical Research Melbourne, VIC domenic.larosa@monash.edu	**Dr. Leo Leader** The University of NSW Sydney, NSW l.leader@unsw.edu.au	**Mr. Christopher Lear** The University of Auckland Auckland, New Zealand christopher.lear@auckland.ac.nz
Dr. Jingang Li MIMR-PHI Institute of Medical Research Melbourne, VIC jingang.li@monash.edu	**Miss. Anqi Li** MIMR-PHI Institute of Medical Research Melbourne, VIC anqi.li@monash.edu	**Dr. Joon Lim** Baker IDI Heart & Diabetes Institute Melbourne, VIC joon.lim@bakeridi.edu.au
Dr. Barbara Lingwood The University of Queensland Brisbane, QLD b.lingwood@uq.edu.au	**Mr. Paul Lombardo** Monash University Melbourne, VIC paul.lombardo@monash.edu	**Ms. Hui Lu** MIMR-PHI Institute of Medical Research Melbourne, VIC hui.lu@monash.edu
Prof. Eugenie Lumbers The University of Newcastle Newcastle, NSW eugenie.lumbers@newcastle.edu.au	**Ms. Nurul Mahizir** The University of Melbourne Melbourne, VIC n.mahizir@student.unimelb.edu.au	**Mr. Jarod Mamrot** MIMR-PHI Institute of Medical Research Melbourne, VIC jared.mamrot@monash.edu
Mr. Yeukai Mangwiro Latrobe University Melbourne, VIC ytmangwiro@students.latrobe.edu.au	**Dr. Kathryn Martinello** Women's and Children's Hospital Melbourne, VIC kathryn.martinello@gmail.com	**Ms Megumi Matsumodo** Saitama Medical University Saitama, Japan whitefhy@yahoo.co.jp
Dr. Courtney McDonald MIMR-PHI Institute of Medical Research Melbourne, VIC courtney.mcdonald@monash.edu	**Miss. Erin McGillick** The University of South Australia Adelaide, SA erin.mcgillick@mymail.unisa.edu.au	**Miss. Kirsten McInerney** The University of Newcastle Newcastle, NSW kirsten.mcinerney@uon.edu.au
Dr. Suzie Miller MIMR-PHI Institute of Medical Research Melbourne, VIC suzie.miller@monash.edu	**A/Prof. Karen Moritz** The University of Queensland Brisbane, QLD k.moritz@uq.edu.au	**Prof. Margaret Morris** The University of New South Wales Sydney, NSW **m.morris@unsw.edu.au**
Prof. Janna Morrison The University of South Australia Adelaide, SA janna.morrison@unisa.edu.au	**A/Prof. Tim Moss** MIMR-PHI Institute of Medical Research Melbourne, VIC tim.moss@monash.edu	**Ms. Alison Moxham** MIMR-PHI Institute of Medical Research Melbourne, VIC **alison.moxham@monash.edu**
Dr. Beverly Muhlhausler The University of Adelaide Adelaide, SA beverly.muhlhausler@adelaide.edu.au	**Mr Shinji Nakamura** MIMR-PHI Institute of Medical Research Melbourne, VIC shinji980@hotmail.com	**Prof. Christopher Nolan** The Canberra Hospital Canberra, ACT christopher.nolan@anu.edu.au
Ms. Sharon Ololo MIMR-PHI Institute of Medical Research Melbourne, VIC sharonololo@gmail.com	**A/Prof. Sandra Orgeig** The University of South Australia Adelaide, SA sandra.orgeig@unisa.edu.au	**Ms. Paris Papagianis** MIMR-PHI Institute of Medical Research Melbourne, VIC paris.papagianis@monash.edu

Miss. Madison Paton MIMR-PHI Institute of Medical Research Melbourne, VIC madison.paton@monash.edu	**Prof. Jane Pillow** The University of Western Australia Perth, WA jane.pillow@uwa.edu.au	**Dr. Julia Pitcher** The University of Adelaide Adelaide, SA julia.pitcher@adelaide.edu.au
Dr. Graeme Polglase MIMR-PHI Institute of Medical Research Melbourne, VIC graeme.polglase@monash.edu	**Prof. Susan Prescott** The University of Western Australia Perth, WA susan.prescott@uwa.edu.au	**Dr. Kristy Pringle** The University of Newcastle Newcastle, NSW kirsty.pringle@newcastle.edu.au
Ms. Shalini Rajakaruna MIMR-PHI Institute of Medical Research Melbourne, VIC shalini.rajakaruna@monash.edu	**Ms. Shreya Rana** MIMR-PHI Institute of Medical Research Melbourne, VIC shreya.rana@monash.edu	**Dr. Anna Rocha** MIMR-PHI Institute of Medical Research Melbourne, VIC anna.rocha@monash.edu
Ms. Karyn Rodgers MIMR-PHI Institute of Medical Research Melbourne, VIC karyn.rodgers@monash.edu	**Ms. Dana Ryan** Monash University Melbourne, VIC danica.vojisavljevic@monash.edu	**Miss. Riana Samuel** MIMR-PHI Institute of Medical Research Melbourne, VIC rsam16@student.monash.edu
Mr. Richard Schlegel The University of Queensland Brisbane, QLD richard.schlegel@uqconnect.edu.au	**Mr. Bennet Seow** Monash University Melbourne, VIC bennet.seow@monash.edu	**Ms. Nikeh Shariatian** MIMR-PHI Institute of Medical Research Melbourne, VIC nikeh.shariatian@mimr-phi.org
Ms. Julia Shaw The University of Newcastle Newcastle, NSW julia.shaw@uon.edu.au	**A/Prof. Joe Smolich** Murdoch Children's Research Institute Melbourne, VIC joe.smolich@mcri.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	**Dr. Amy Sutherland** MIMR-PHI Institute of Medical Research Melbourne, VIC amy.sutherland@monash.edu	**Prof. Keiji Suzuki** Tokai University Kanagawa, Japan dkswnd@tokai-u.jp
A/Prof. David Todd Centenary Hospital Canberra, ACT david.todd@act.gov.au	**Dr. Mary Tolcos** MIMR-PHI Institute of Medical Research Melbourne, VIC mary.tolcos@mimr-phi.org	**Ms. Stacey Vranas** Monash University Melbourne, VIC stacey.Vranas@monash.edu
Ms. Amanda Vrselja Monash University Melbourne, VIC amanda.vrselja@monash.edu	**Prof. David Walker** MIMR-PHI Institute of Medical Research Melbourne, VIC david.walker@monash.edu	**Dr. Megan Wallace** MIMR-PHI Institute of Medical Research 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 Wooldridge** The University of Adelaide Adelaide, SA amy.wooldridge@adelaide.edu.au

Prof. Ian Wright The University of Newcastle Newcastle, NSW ian.wright@newcastle.edu.au	**Ms. Lisa Yamada** Mater Research Institute South Brisbane, QLD lisa.Yamada@mater.uq.edu.au	**Dr. Tamara Yawno** MIMR-PHI Institute of Medical Research Melbourne, VIC tamara.yawno@monash.edu
Dr. Stephanie Yiallourou MIMR-PHI Institute of Medical Research Melbourne, VIC stephanie.yiallourou@monash.edu	**Ms. Jia Yin Soo** The University of South Australia Adelaide, SA soojy002@mymail.unisa.edu.au	**Ms. Valerie Zahra** MIMR-PHI Institute of Medical Research Melbourne, VIC valerie.zahra@monash.edu
Prof. Tamás Zakár Hunter Medical Research Institute Newcastle, NSW tamas.Zakar@newcastle.edu.au	**Miss. Diana Zanfirache** The University of Queensland Brisbane, QLD diana.zanfirache@uqconnect.edu.au	