FETAL AND NEONATAL WORKSHOP
OF AUSTRALIA AND NEW ZEALAND
31st Annual Meeting

University House
The Australian National University
Canberra, ACT
30-31 March, 2017

2017 Organising Committee

Principal Organising Committee
Dr Robert De Matteo

Local Organising Committee
Prof Alison Kent
A/Prof David Todd

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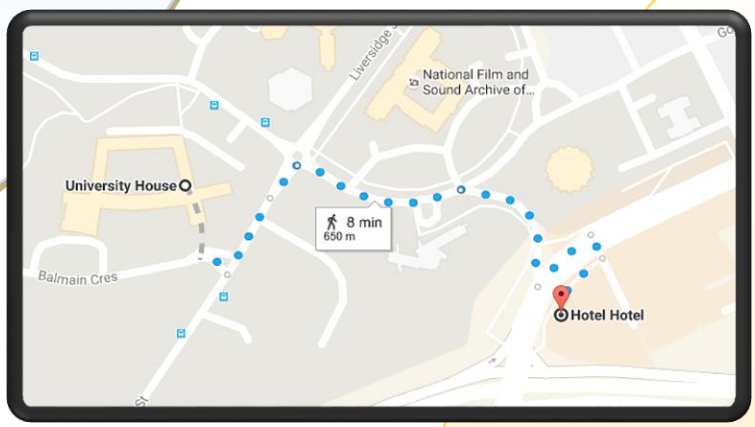
The FNWANZ is proudly affiliated with the Perinatal Society of Australia and New Zealand

Programme Outline - 2017

University House
The Australian National University, Canberra, ACT

THURSDAY 30TH MARCH	
9.30am-10.25am	**Registration**
10.25am-10.30am	**Official Opening**
10.30am-11.45am	**Session 1**
11.45am-12.15pm	**Morning Tea**
12.15pm-1.30pm	**Session 2**
1.30pm-2.30pm	**Lunch**
2.30pm-3.45pm	**Session 3**
3.45pm-4.15pm	**Afternoon Tea**
4.15pm-5.30pm	**Session 4**
7.00pm-11.30pm	**Workshop Dinner**

Hotel Hotel
25 Edinburgh Ave, Canberra



FRIDAY 31ST MARCH	
9.30am-10.00am	**Registration**
10.00am-11.15am	**Session 5**
11.15am-11.45am	**Morning Tea**
11.45am-1.00pm	**Session 6**
1.00pm-2.00pm	**Lunch**
2.00pm-3.15pm	**Session 7**
3.15pm-3.45pm	**Afternoon Tea**
3.45pm-4.45pm	**Session 8**
5.00pm-5.15pm	
Presentation of student prizes, FNW 2018, Close of Workshop	

FNWANZ Scientific Programme - 2017

DAY 1 - THURSDAY 30th March

Registration: 9.30-10.30am

Hons=Honours, E=Early PhD, L=Late PhD, ECR=Early Career Researcher

Session 1: Chairs – Alison Kent and Jon Hirst			
10.30am	**A1**	Amy Wooldridge (ECR)	Effects of postnatal glucocorticoid exposure on the immune system of preterm lambs
10.45am	**A2**	Shreya Rana (E)	Effects of maternal immune activation on structural brain growth
11.00am	**A3**	Kate Goasdoué (E)	Alterations in BBB and inflammatory markers in piglets with seizures following an hypoxic ischaemic insult
11.15am	**A4**	Ella Edward (Hons)	Postnatal influences on gut function and histopathology in LPS exposed preterm lambs
11.30am		*General discussion*	

Morning tea: 11.45am-12.15pm

Session 2: Chairs – David Todd and Julia Pitcher			
12.15pm	**A5**	Alistair Gunn	Head cooling for 48 hours is insufficient for optimal neuroprotection after global cerebral ischemia in term-equivalent fetal sheep
12.30pm	**A6**	Stephanie Miller (L)	Developmental expression of $GABA_A$ α_1 and α_3 subunits in the pig brain
12.45pm	**A7**	Julie Wixey (ECR)	Anti-inflammatory treatment to reduce brain injury in the growth restricted neonate
1.00pm	**A8**	Lara Rijkmans (E)	Neuropathology of the growth restricted spiny mouse fetus
1.15pm		*General discussion*	

Lunch: 1.30pm-2.30pm

Session 3: Chairs – Graeme Polglase and Megan Sutherland			
2.30pm	**A9**	Harleen Kaur (E)	The effect of maternal intake of sucrose and HFCS-55 during gestation and lactation on lipogenic gene expression in offspring at 3 and 12 weeks of age
2.45pm	**A10**	Sonja Brennan (E)	The renal parenchyma – Evaluation of a novel ultrasound measurement to assess fetal renal development
3.00pm	**A11**	Emma Buckels (L)	Does maternal sildenafil citrate treatment in growth-restricted ovine pregnancies protect the pancreas from beta cell loss?
3.15pm	**A12**	Mikee Inocencio (E)	Nitric oxide as a potential therapeutic against cardiovascular dysfunction in fetal growth restricted fetuses
3.30pm		*General discussion*	
Afternoon tea: 3.45pm-4.15pm			
Session 4: Chairs – Laura Bennet and Michael Stark			
4.15pm	**A13**	Martha Calalang (Hons)	Umbilical cord blood as therapy for preterm vascular injury
4.30pm	**A14**	Joseph Smolich	Systemic flow increases after ductal closure are related to redistribution of arterial reservoir discharge in preterm newborn lambs
4.45pm	**A15**	Amanda Vrselja (L)	The effect of postnatal steroids on heart structure in preterm lambs
5.00pm	**A16**	Ryley Macrae (L)	Dexamethasone increases the stiffness of the developing lamb aorta
5.15pm		*General discussion*	

7.00pm – Workshop Dinner: Hotel Hotel, 25 Edinburgh Ave, Canberra

FNWANZ Scientific Programme - 2017

DAY 2 - FRIDAY 31st March

Registration: 9.30-10.00am

Hons=Honours, E=Early PhD, L=Late PhD, ECR= Early Career Researcher

Session 5: Chairs – Rosemary Horne and Leo Leader			
10.00am	**A17**	Samantha Rodrigues (E)	(Pro)renin receptor knockdown reduces rate of cell proliferation in a first trimester extravillous trophoblast cell line, HTR-8/SVneo
10.15am	**A18**	Anya Arthurs (E)	Oxygen regulation of placental miRNA expression
10.30am	**A19**	Saije Morosin (E)	The role of the prorenin receptor ((P)RR) and soluble prorenin receptor (s(P)RR) in syncytialisation
10.45am	**A20**	Nadia Bellofiore (L)	Behavioural changes in the spiny mouse are indicative of pre-menstrual syndrome
11.00am	*General discussion*		

Morning tea: 11.15am-11.45am

Session 6: Chairs – Alistair Gunn and Jane Pillow			
11.45am	**A21**	Jonathan Davis (ECR)	Postnatal dexamethasone effect on preterm lamb lung development
12.00pm	**A22**	Erin McGillick (ECR)	Elevated airway liquid volumes at birth: A potential cause of transient tachypnea of the newborn
12.15pm	**A23**	Kyra Chan (E)	Isolating effects of the initiation of ventilation on the preterm lamb brain
12.30pm	**A24**	Shigeo Yamaoka	Understanding the effect of transient tachypnea of the newborn on cardiorespiratory function in premature newborn lambs
12.45pm	*General discussion*		

Lunch: 1.00pm-2.00pm

Session 7: Chairs – Tamás Zakár and Hayley Dickinson			
2.00pm	**A25**	Paris Papagianis (L)	Viability and function of human amnion epithelial cells is not altered by temperature
2.15pm	**A26**	Tayla Penny (E)	Does administration of umbilical cord blood cells ameliorate long term behavioural effects following neonatal hypoxic ischemic brain injury?
2.30pm	**A27**	Laura Bennet	Delayed intranasal infusion of human amnion epithelial cells improves white matter maturation after asphyxia in preterm fetal sheep
2.45pm	**A28**	Madison Paton (L)	Umbilical cord blood therapy modulates LPS-induced brain injury in preterm fetal sheep
3.00pm	*General discussion*		

Afternoon tea: 3.15pm-3.45pm

Session 8: Chairs – Tim Moss and Kirsty Pringle			
3.45pm	**A29**	Jago Van Dam (E)	Reduced cortical excitability, neuroplasticity and cortisol in children exposed to gestational diabetes in utero: Is metformin neuroprotective
4.00pm	**A30**	Nathanael Yates (ECR)	Developmental Vitamin D deficiency and autism spectrum disorder phenotypes in male rats
4.15pm	**A31**	Kelsee Shepherd (E)	Cardiorespiratory events in preterm infants: Effect of sleeping position, sleep state and postnatal age
4.30pm	*General discussion*		
5.00pm	**Presentation of Prizes**		
5.05pm	**Fetal and Neonatal Workshop 2018**		
5.10pm	**Close of Workshop**		

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

Terms of reference

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

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

Session 1

Chairs – Alison Kent and Jon Hirst			
10.30am	**A1**	**Amy Wooldridge (ECR)**	**Effects of postnatal glucocorticoid exposure on the immune system of preterm lambs**
10.45am	**A2**	**Shreya Rana (E)**	**Effects of maternal immune activation on structural brain growth**
11.00am	**A3**	**Kate Goasdoué (E)**	**Alterations in BBB and inflammatory markers in piglets with seizures following an hypoxic ischaemic insult**
11.15am	**A4**	**Ella Edward (Hons)**	**Postnatal influences on gut function and histopathology in LPS exposed preterm lambs**
11.30am	**General discussion**		

Effects of postnatal glucocorticoid exposure on the immune system of preterm lambs

Amy Wooldridge[1,2], Jane Pillow[1,2], Siavash Ahmadi-Noorbakhsh[2], Andrew Currie[3]

[1]School of Human Sciences, [2]Centre for Neonatal Research and Education, School of Paediatrics and Child Health, Crawley, University of Western Australia; [3]School of Veterinary and Life Sciences, Murdoch University, Murdoch, Australia.
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Background: Preterm birth currently affects ~8 % of neonates born in Australia. Postnatal steroids are anti-inflammatories used in clinical practice to accelerate weaning of neonates from mechanical ventilation and to either prevent or rescue infants with severe bronchopulmonary dysplasia from impending respiratory failure. Whilst the immunosuppressive nature of postnatal exogenous glucocorticoid exposure is well-known, recent studies have found that this treatment can alter human helper T1-T2 balance, restored during adolescence.[1-2]

Aims: To investigate changes in markers and mediators of inflammation associated with postnatal glucocorticoid exposure.

Methods: Date-mated merino ewes were administered antenatal betamethasone (5.7 mg/dose, i.m.) at 24 h intervals in the two days prior to caesarean section delivery. Lambs were delivered at day 129 of gestation (term ~150 days) and quasi-randomised to receive postnatal dexamethasone (0.15 mg/kg x 3 d, 0.1 mg/kg x 2 d, 0.05 mg/kg x 2 d) or saline as an experimental control. The lambs received 24 hour care within the Preclinical Intensive Care Research Unit during at least the first week of life. At two months corrected postnatal age, the lambs were euthanised and the thymus, spleen and posterior mediastinal and ileal lymph nodes were removed and sampled for histological and protein analyses. These samples are being analysed in parallel with samples from similarly-treated lambs euthanised at day 7 postnatal age.

Results: Absolute and relative organ weights did not differ between groups. Analysis of thymic tissue is currently in progress. A detailed plan for tissue analysis will be presented at the Workshop.

Conclusions: It is expected that immune differences seen at 7 days postnatal age will be less prominent at 2 months postnatal age. This study will increase our understanding of perinatal immune programming.

1. Wolbeek et al. (2015) Brain, Behavior, and Immunity 45:128-138
2. Karemaker et al. (2008) Pediatrics 121:e870-e878

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Effects of maternal immune activation on structural brain growth

Shreya Rana[1], Rosa Shishegar[2], Mary Tolcos[3], David Walker[3]

[1]The Ritchie Centre, Hudson Institute of Medical Research, Clayton, Australia; [2]Department of Electrical & Electronic Engineering, University of Melbourne, Melbourne, Australia; [3]School of Health and Biomedical Sciences, RMIT University, Bundoora, Australia.
Shreya.Rana@hudson.org.au

Background: Maternal immune activation (MIA) by an infection during pregnancy results in adverse neurobehavioural outcomes including autism and schizophrenia. Animal studies on the neurological impacts of MIA have largely focussed on behavioural, molecular and histological changes rather than on the integrated growth of the brain. Here we aim to establish a preclinical model of MIA using the spiny mouse to study the impact of MIA at different gestational times on brain growth. The spiny mouse is a precocious species in which the adrenal hormones cortisol and dehydroepiandrosterone (DHEA) influence placental and fetal brain development, as occurs in human pregnancy.

Aims/Hypothesis: We aim to identify the effect of gestational timing of MIA on regional brain growth. Based on current knowledge of spiny mouse neurodevelopment and fetal neurological structures affected by MIA we hypothesise that late gestation exposure to MIA will affect hippocampal growth, while early and mid gestation exposure to MIA will predominantly affect cortical grey matter and deep grey matter volumes respectively.

Methods: Pregnant spiny mice are given either 5mg/kg Poly I:C to induce a maternal immune response or saline (control) s.c at 10, 20, or 30 days gestation (dGA; term = 39dGA). Fetal pups are delivered at 37dGA and the brains are imaged using a 9.4T MRI scanner, and analysed using 3D digital rendering software to determine volumes of the following brain regions: neocortical grey matter, hippocampus, deep grey matter and midbrain.

Results: We have developed an accurate and unbiased approach to quantifying regional brain volumes by 3-dimensionally reconstructing brain regions in our cohort of offspring exposed to Poly I:C at 30dGA.

Conclusions: We expect that late gestation MIA will lead to a reduction in fetal hippocampal volume. This information will be used to guide histological characterisation of specific brain regions for our entire experimental cohort (i.e. when Poly I:C is given at 10, 20 or 30 days gestation) to provide a better understanding of the influence of gestational timing of MIA on fetal brain growth.

Alterations in BBB and inflammatory markers in piglets with seizures following an hypoxic ischaemic insult

Kate Goasdoué, Stephanie M Miller, Kirat Chand, Julie Wixey, Paul Colditz, Tracey Björkman

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Background: The blood-brain barrier (BBB) is an integral part of a functioning CNS. A fully functional and intact BBB is vital for correct neurological function. Disruption of the BBB has been shown to prime the brain toward excessive excitation and seizures. The primary aetiology of neonatal seizures is hypoxic-ischaemic encephalopathy – a disease in which BBB disruption is also a hallmark. Pro-inflammatory cytokines have implicated in BBB disruption following hypoxic-ischaemic-related seizures.

Aims/Hypothesis: The hypothesis is that levels of inflammation and BBB disruption will be significantly different in animals with seizure following an hypoxic-ischaemic (HI) insult than animals without seizures following HI. We aim to investigate this by analysing mRNA expression of inflammatory markers and genes associated with BBB integrity via RT-PCR in cortex and hippocampus of piglets who have undergone HI.

Methods: A neonatal piglet model of hypoxia-ischaemia with spontaneous seizures will be utilised. Anaethetised animals undergo a 30min HI-insult which includes a period of at least 10min of hypotension. Animals are culled at 72h post-HI, brain are removed and snap frozen in liquid nitrogen. RNA is extracted from frozen brain tissue (Macherey Nagel). RT-PCR Quantitative polymerase chain reaction (qPCR) was performed using SYBR green PCR master mix (Invitrogen), 2 μM of gene-specific primers (Sigma Aldrich), 50 ng of cDNA and a Rotor-Gene 6000 (Corbett Life Science).

Results: The presence of seizure is associated with differential expression of genes associated with BBB integrity and inflammatory markers. Most notably, HI animals with seizure have an upregulation of MMP2, CLDN5, IL-6, IL-8, and TGFβ, whilst those genes in animals without seizure are downregulated.

Conclusions: Increasing our understanding of the mechanisms involved in seizure generation and progression in the neonate is vital because neonatal seizures often do not respond to treatment and are often symptomatic of CNS pathology. Investigating the BBB in these conditions is a major untapped area of research and potential avenue for novel treatments.

Postnatal influences on gut function and histopathology in LPS exposed preterm lambs

Ella Edward, Jane Pillow, Amy Wooldridge

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Background: Proinflammatory fetal responses to intra-amniotic (IA) lipopolysaccharide (LPS) mimic the systemic fetal inflammatory response to chorioamnionitis. IA LPS also causes local adverse effects on the fetal intestine (Wolfs et al., 2009).

Aims/Hypothesis: To determine if adverse effects of antenatal LPS on the preterm lamb intestine persist after exposure to enteral feeds and postnatal intensive care, and to determine if postnatal glucocorticoid modulates the response of the immature intestine to antenatal LPS.

Methods: Date-mated ewes received IA injection of LPS (4 mg) or equivalent volume of saline (SAL) 2 d prior to delivery at 129 d gestation. Preterm lambs received postnatal saline or a low dose (0.75 mg/kg) tapered dexamethasone course over 7 days resulting in 4 postnatal groups: SAL/SAL (n= 11); SAL/DEX (n= 9); LPS/SAL (n= 8); LPS/DEX (n= 9). Preterm lambs were euthanized on postnatal day 7. Naive 136 d gestation fetal lambs were used as maturational controls (FC: n=7). Feed tolerance was assessed as total enteral fluid intake. Mucosal morphology and integrity was examined by measurements of: villus height, crypt depth, and semi-quantitative index of mucosal sloughing. Intestinal inflammation was determined from CD45+ cell count in the jejunum and distal ileum. Plasma concentration of glucagon-like peptide 2 (GLP-2) and gastrin was measured by ELISA.

Results: Enteral fluid intake increased steadily over 7 days. Villus height was not affected by LPS or dexamethasone, but crypt depth was increased by dexamethasone ($p= 0.011$). SAL/SAL control lambs had increased villus sloughing in the ileum ($p=0.017$), increased CD45+ cell count in the jejunum ($p= 0.037$) and ileum ($p= 0.007$), and increased plasma gastrin concentration ($p<0.001$) relative to fetal control lambs. Villus sloughing, CD45+ cell count, and plasma concentration of gastrin and GLP-2 were not altered by LPS or dexamethasone exposure.

Conclusions: Prematurity and early exposure to enteral feeds compromised intestinal function and mucosal integrity, and caused inflammation. Postnatal factors were predominant in determining gut outcome.

Wolfs, T et al (2009). Endotoxin induced chorioamnionitis prevents intestinal development during gestation in fetal sheep. PLoS One. 2009 Jun 8;4(6)

Session 2

Chairs – David Todd and Julia Pitcher			
12.15pm	**A5**	**Alistair Gunn**	**Head cooling for 48 hours is insufficient for optimal neuroprotection after global cerebral ischemia in term-equivalent fetal sheep**
12.30pm	**A6**	**Stephanie Miller (L)**	**Developmental expression of $GABA_A$ α_1 and α_3 subunits in the pig brain**
12.45pm	**A7**	**Julie Wixey (ECR)**	**Anti-inflammatory treatment to reduce brain injury in the growth restricted neonate**
1.00pm	**A8**	**Lara Rijkmans (E)**	**Neuropathology of the growth restricted spiny mouse fetus**
1.15pm	**General discussion**		

Head cooling for 48 hours is insufficient for optimal neuroprotection after global cerebral ischemia in term-equivalent fetal sheep

Joanne O. Davidson, Vittoria Draghi, Sean Whitham, Simerdeep Dhillon, Giudo Wassink, Laura Bennet, Alistair J. Gunn

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Background: Therapeutic hypothermia for 72 h reduces death and disability in neonates with hypoxic-ischemic encephalopathy. However, it is surprisingly unclear whether 72 h of cooling is essential for optimal neuroprotection. Cooling for 5 days does not further improve protection and may increase mortality, while in adult rodents mild to moderate hypothermia for 48 h after ischemia was associated with durable protection. If a shorter duration of cooling was effective, then it would help reduce the burden of treatment.

Aims/Hypothesis: To determine the relative neuroprotective efficacy with delayed head cooling for 48 h compared to 72 h.

Methods: Term-equivalent fetal sheep (0.85 gestation) received 30 min of sham ischemia (n = 8) or ischemia induced by bilateral carotid artery occlusion followed by normothermia (n = 8) or head cooling started 3 h after ischemia, and continued for either 48 h (n = 8) or 72 h (n = 8). Fetuses were killed 7 days after ischemia.

Results: Cerebral ischemia was associated with profound loss of EEG power after 7 days recovery, with severe loss of neurons in the cortex and hippocampus, loss of oligodendrocytes in the intragyral and periventricular white matter tracts and induction of Iba-1-positive microglia. Although head cooling for 48 h was associated with improved outcomes compared to normothermia, compared to cooling for 72 h, it was associated with less recovery of EEG power ($P < 0.05$), less improvement in neuronal survival in the parasagittal cortex and the CA4 region of the hippocampus ($P < 0.05$), less attenuation of microglial induction ($P < 0.05$), but a similar partial improvement in numbers of oligodendrocytes ($P < 0.05$). Rewarming at 48 hours was associated with a striking deterioration in EEG power.

Conclusions: Head cooling for 48 h is partially neuroprotective, but is suboptimal compared to cooling for 72 h after global cerebral ischemia in fetal sheep. The close association between rewarming at 48 h and subsequent deterioration in EEG power strongly denotes ongoing encephalopathic processes between 48 and 72 h after ischemic injury.

Developmental expression of $GABA_A$ α_1 and α_3 subunits in the pig brain

Stephanie M Miller, Paul B Colditz, S. Tracey Björkman

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Background: GABA is a major neurotransmitter in the mammalian brain. In the mature brain GABA exerts inhibitory actions via the $GABA_A$ receptor ($GABA_AR$), however in the immature brain GABA provides much of the excitatory drive. $GABA_AR$ α_2 and α_3-subunit mRNA are abundantly expressed in the immature rodent cortex hippocampus and thalamus, with α_1-subunit expression increasing postnatally to be the predominant α-subunit in the mature brain (Laurie et al 1992). We have recently reported a significant upregulation of α_1 and downregulation of α_3 protein expression with increasing age, in various regions of the pig brain (*in press*).

Aims/Hypothesis: To investigate differences in protein expression of the developmental regulated $GABA_A$ receptor α_1 and α_3 subunits in cellular fractions of the pig brain across four selected ages (two preterm and two postnatal).

Methods: Piglet brain tissue was obtained from preterm animals at 91d and 100d gestation (term = 115d); and from P4 and adult (n=5 per age). Regions of parietal cortex, hippocampus and thalamus were frozen prior to sample preparation into nuclear, membrane and cytosolic fractions by ultracentrifugation. West blot analysis was used to investigate the protein expression of the $GABA_A$ receptor α_1 and α_3 subunits.

Results: With increasing age there were increases on membrane $GABA_A$ α_1 expression in the parietal cortex and hippocampus, while small reductions were observed in thalamic samples. Cytosolic α_1 expression increased with age in the three regions investigated. $GABA_A$ α_3 was highly expressed in the nuclear and membrane fractions of preterm brain, with a significant peak in membrane α_3 at 100d.

Conclusions: We examined the subcellular expression of two of the major $GABA_A$ α-subunit proteins at four selected ages. Nuclear and membrane expression of α_3 decreased with age, while α_1 expression increased. $GABA_A$ α_1 expression is associated with faster inhibitory kinetics, and the upregulation of membrane α_1 expression is consistent with maturation of GABAergic inhibition.

Anti-inflammatory treatment to reduce brain injury in the growth restricted neonate

Julie Wixey, Kishen Sukumar, Stephanie Miller, Kate Goasdoué, Paul Colditz, Tracey Björkman, Kirat Chand

Perinatal Research Centre, UQ Centre for Clinical Research, The University of Queensland, Herston, Australia.
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Background: The fetal brain is particularly vulnerable to the effects of intrauterine growth restriction (IUGR) which can increase the risk of long-term neurological disorders such as cerebral palsy and psychiatric illness. Limited treatments are available to prevent brain injury in the IUGR neonate. Therefore research must focus on brain injury mechanisms in the IUGR brain to best determine treatment options. It is becoming apparent that inflammation is a key player in the IUGR brain. Whether treatment with an anti-inflammatory can prevent inflammation and concurrent brain injury is to be determined.

Aims/Hypothesis: We hypothesize inflammation is prevalent in the IUGR piglet brain and administration of the anti-inflammatory ibuprofen, will reduce the inflammatory response.

Methods: Newborn IUGR (<5th centile) and age-matched normally grown control piglets were brought to Herston Medical Research Centre to be monitored, fed and cared for until euthanasia on postnatal day 4. The treatment group received Ibuprofen (20mg/kg/day on day 1 and 10mg/kg/day on days 2 and 3) in the piglet formula during the morning feed each day. The cellular markers of inflammation, microglia (Iba-1) and astrocytes (GFAP), were examined using immunohistochemistry.

Results: A significant increase in microglia and changes to cellular morphology of glial cells were apparent in the IUGR piglet cortex on postnatal day 4. Administration of ibuprofen decreased the number of reactive/active glial cells in the IUGR piglet brains.

Conclusions: Activated microglia and reactive astrocytes are known to exacerbate neuronal injury in the neonate. They release large amounts of proinflammatory cytokines perpetuating detrimental effects on neurons. Future studies within our group will determine whether ibuprofen treatment can also prevent neuronal injury and improve neurological outcome in IUGR neonates.

Neuropathology of the growth restricted spiny mouse fetus

Lara Rijkmans[1,2], Hayley Dickinson[1], Suzie Miller[1], Nadia Hale[1], David Walker[2], Nadia Hale[1], Mary Tolcos[2]

[1]Embryology & Placental Biology Research Group, The Ritchie Centre, Hudson Institute of Medical Research, Victoria, Australia; [2]Developmental Brain Injury & Repair Research Group, School of Health & Biomedical Sciences, RMIT University, Victoria, Australia.
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Background: Intrauterine growth restriction (IUGR), is a condition occurring in approximately 7% of the obstetric population. Rodent studies have examined the neuropathology of IUGR, however the timing of brain development, hormone profiles and induction of IUGR via partial uterine occlusion in the recently developed spiny mouse model (Dickinson et al, 2017) may more closely mimic the human condition, hence the need determine its appropriateness for interventional studies.

Aims/Hypothesis: To determine whether IUGR induced by restricting the gestational increase of uterine blood flow in the spiny mouse results in fetal brain injury and/or alterations in fetal brain development.

Methods: At 27 days gestational age (dg; term is 38dg), a piece of silastic tubing was placed around the left uterine artery to prevent the further increase in diameter of the artery with advancing gestation. Control pregnancies were generated from sham surgeries without placement of the tubing. At 37dg, the brains (IUGR, n=18 and Control, n=7) were fixed and sagittal sections stained histologically (H&E) for morphological analysis of the corpus callosum, and immunostained to identify myelinated fibres (myelin basic protein; MBP), oligodendrocytes (oligodendrocyte transcription factor 2; Olig2), astrocytes (glial fibrillary acidic protein; GFAP) and microglia (ionized calcium binding adapter molecule-1; Iba-1). Data are presented as mean±SEM and significance is $p<0.05$.

Results: In IUGR vs control fetuses there was no difference in: a) the total cross sectional area (mm^2) of the cerebral white matter or grey matter; b) width of the corpus callosum (μm); c) areal density (cells/mm^2) of microglia, oligodendrocytes, or astrocytes; and d) the area covered (%) by MBP- or GFAP-immunoreactivity. There was a positive correlation between brain weight and area coverage of GFAP-immunoreactivity in the corpus callosum, but not with any other outcome measures.

Conclusions: The results of this study indicate that IUGR induced by partial uterine artery occlusion in the spiny mouse fetus does not alter cerebral development or result in overt injury to the corpus callosum.

Dickinson H et al J DoHaD (in press)

Session 3

Chairs – Graeme Polglase and Megan Sutherland			
2.30pm	A9	Harleen Kaur (E)	The effect of maternal intake of sucrose and HFCS-55 during gestation and lactation on lipogenic gene expression in offspring at 3 and 12 weeks of age
2.45pm	A10	Sonja Brennan (E)	The renal parenchyma – Evaluation of a novel ultrasound measurement to assess fetal renal development
3.00pm	A11	Emma Buckels (L)	Does maternal sildenafil citrate treatment in growth-restricted ovine pregnancies protect the pancreas from beta cell loss?
3.15pm	A12	Mikee Inocencio (E)	Nitric oxide as a potential therapeutic against cardiovascular dysfunction in fetal growth restricted fetuses
3.30pm	General discussion		

The effect of maternal intake of sucrose and HFCS-55 during gestation and lactation on lipogenic gene expression in offspring at 3 and 12 weeks of age.

Harleen Kaur[1], Carla Toop[1], Beverly S. Muhlhausler[1,2], Sheridan Gentili[1]

[1]Address-School of Pharmacy and Medical Sciences, Sansom Institute for Health Research, University of South Australia, Adelaide, Australia; [2]Address- FOODplus Research Centre, School of Agriculture, Food and Wine, The University of Adelaide, Adelaide, Australia.
harleen.kaur@adelaide.edu.au

Background: Consumption of sucrose and high fructose corn syrup-55 (HFCS-55) have been implicated as important contributors to the global obesity and type 2 diabetes epidemics in children and adults, however little is known of the effects of exposure to these sugars before birth or in the early postnatal period on future metabolic health. We have previously found that prenatal exposure to sucrose or HFCS-55 resulted in increased liver weight in rat pups at birth, and altered hepatic fat content and composition at weaning at 12 weeks of age, however effects on hepatic metabolism are unknown.

Objective: To investigate and compare the effects of maternal consumption of sucrose and HFCS-55 during pregnancy and lactation on hepatic expression of lipogenic genes in the offspring.

Methods: Liver samples were collected from offspring exposed to a maternal control, 10% sucrose, or 10% HFCS-55 supplemented diet at 3 weeks (control, n=16; sucrose, n=22; HFCS, n=6) and 12 weeks (control, n=16; sucrose, n=10; HFCS, n=16) of age. Hepatic expression of acetyl-CoA carboxylase-1, apolipoprotein B100, carbohydrate response element binding protein (ChREBP), fatty acid synthase, stearoyl CoA desaturase-1 and sterol regulatory element binding protein-1c (SREBP-1c) was determined by qRT-PCR.

Results: Maternal sucrose consumption was associated with a persistent increase in SREBP-1c expression at 3 (P=0.01) and 12 weeks (P=0.03) compared to control and HFCS groups. There were no differences in the expression of other hepatic lipogenic genes at either 3 or 12 weeks of age.

Conclusions: The upregulation of SREBP-1c mRNA in offspring exposed to maternal sucrose consumption during pregnancy and lactation suggests that hepatic lipid synthesis may be enhanced in these offspring, which in turn has the potential to promote hepatic lipid accumulation and place the offspring at risk of non-alcoholic fatty liver disease and other metabolic complications later in life.

The renal parenchyma – Evaluation of a novel ultrasound measurement to assess fetal renal development

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Background: Abnormal fetal growth can adversely impact on renal development and is associated with increased risks of developing hypertension and chronic renal disease later in life. A non-invasive, sensitive method of assessing normal and abnormal fetal kidney development is needed. We hypothesise that the renal parenchymal thickness could be used to evaluate the impact of abnormal fetal growth on the developing fetal renal parenchyma.

Aims/Hypothesis: This study using antenatal ultrasound will assess the renal parenchymal growth in a population demonstrating either normal or abnormal growth and determine if abnormal fetal growth has an effect on renal parenchymal thickness. The relationship between renal parenchymal thickness, renal artery Dopplers, other fetal Dopplers and amniotic fluid will also be assessed. Hypotheses are – a) Renal parenchymal thickness is altered in intrauterine growth restricted and large for gestational age fetuses compared to appropriately grown fetuses, b) Fetal parenchymal growth is correlated with fetal Doppler indices and amniotic fluid levels.

Methods: A longitudinal, observational study will be conducted over 12 months, beginning May 2017. Women with an accurately dated, singleton pregnancy will undergo an ultrasound scan every four weeks between 16 and 40 weeks gestation. Outcome measures will be – renal parenchymal thickness, renal volume, fetal growth biometries, amniotic fluid measurement, umbilical artery Doppler, middle cerebral artery Doppler, renal artery Doppler and renal parenchymal echogenicity.

Results: None to date.

Conclusions: The outcome of this study is to have a better measure and understanding of renal growth according to overall fetal development.

Does maternal sildenafil citrate treatment in growth-restricted ovine pregnancies protect the pancreas from beta cell loss?

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Background: Intrauterine growth restriction (IUGR) is associated with decreased insulin secretory capacity in adulthood. Sildenafil citrate is thought to enhance uterine and placental blood flow, and hence nutrient transfer to the fetus, leading to improved growth in IUGR fetal lambs. We therefore investigated whether sildenafil treatment also mitigated the negative effects of IUGR on pancreatic development.

Hypothesis: That sildenafil citrate treatment of ewes carrying IUGR fetuses mitigates the decreased fetal pancreatic islet β-cell mass induced by IUGR.

Methods: Singleton-bearing ewes underwent uterine artery embolisation between 102-107 days' gestational age (dGA). Ewes were treated with a daily infusion of 150 mg sildenafil (IUGR-SC), or water (IUGR-V), subcutaneously over 12 hours via an infusion pump, for 21 days. Control animals remained unembolised and untreated (Control). Fetal tissues were collected at 132-133 dGA. From a subset of animals, three pancreas sections were stained using immunohistochemistry for insulin, glucagon and somatostatin, subsequently undergoing whole-slide imaging. ImageJ was used to analyse 101 ± 3 mm^2 pancreatic tissue per animal. Islet cell mass was calculated as total cell area (μm^2) x pancreas weight (mg) divided by total tissue area (μm^2).

Results: Lamb weight was reduced significantly in IUGR-V (3447 ± 235 g, n=8), but not IUGR-SC (3668 ± 129 g, n=10), compared to Control (4381 ± 247 g, n=9) animals. Pancreas weight relative to body weight was not different amongst groups. Total islet mass was decreased in IUGR-V (77 ± 14 mg, n=6) compared to Control (156 ± 31 mg, n=4, p=0.03), but IUGR-SC (95 ± 14 mg, n=7) was not different compared to either group. This trend was also observed for β-cell mass (Control, 103 ± 16 mg; IUGR-V, 55 ± 16 mg; IUGR-SC, 72 ± 12 mg, p=0.06), whereas IUGR reduced both α- and δ-cell mass, with no effect of sildenafil treatment.

Conclusions: This study suggests that maternal sildenafil treatment may be promising to both increase fetal growth, and potentially mitigate negative effects of IUGR on total islet and β-cell mass. Further gene and protein expression studies of these tissues are in progress.

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

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Background: Fetal growth restriction (FGR) commonly occurs due to inadequate delivery of nutrients to the fetus. To survive FGR infants mount cardiovascular adaptations to increase blood supply to essential organs, resulting in functional and structural cardiovascular changes. Sildenafil increases vasodilation via prevention of nitric oxide breakdown (NO). Maternal sildenafil administration may improve fetal growth via increased placental vasodilation. We have shown that FGR fetal lambs have a unique response to NO and it is therefore crucial we determine the effect that sildenafil exposure has on the already altered vasculature of the fetus.

Aims/Hypothesis: To determine the effect of maternal sildenafil administration has on fetal size, weight, carotid and femoral blood flow and blood pressure during gestation and investigate effect on vascular structure and function. We hypothesize that sildenafil will improve growth and cardiovascular function of FGR lambs.

Methods: Preterm lambs (0.6 gestation) underwent sterile surgery to induce FGR by single umbilical artery ligation (SUAL, FGR) or sham surgery (control, AG). Fetal catheters were implanted into the right femoral artery and flow probes placed on the left femoral and carotid arteries. Ewes were randomly allocated receive continuous i.v infusion of saline or sildenafil (20ml at 2mg/ml per day) 3 days post-surgery. Physiological recordings were taken on alternate days. At 0.83 gestation lambs were immediately euthanized for collection of vessels. Sections were immediately frozen and fixed for structural and molecular analysis.

Results: SUAL resulted in asymmetrical growth, irrespective of treatment. FGR + sildenafil lambs were smaller, with a larger ratio of brain sparing compared to FGR + saline groups. Blood pressure was lower in sildenafil treated lambs compared to saline treated from day one of infusion. Femoral flow increased similarly in both FGR groups compared to AG. Femoral vascular resistance decreased the sildenafil group compared to the saline group Carotid vascular resistance and blood flow was not different between any groups.

Conclusions: Sildenafil treatment of FGR lambs does not improve growth or cardiovascular function and may exacerbate the consequences of FGR.

Session 4

Chairs – Laura Bennet and Michael Stark			
4.15pm	**A13**	**Martha Calalang (Hons)**	**Umbilical cord blood as therapy for preterm vascular injury**
4.30pm	**A14**	**Joseph Smolich**	**Systemic flow increases after ductal closure are related to redistribution of arterial reservoir discharge in preterm newborn lambs**
4.45pm	**A15**	**Amanda Vrselja (L)**	**The effect of postnatal steroids on heart structure in preterm lambs**
5.00pm	**A16**	**Ryley Macrae (L)**	**Dexamethasone increases the stiffness of the developing lamb aorta**
5.15pm	**General discussion**		

Umbilical cord blood as therapy for preterm vascular injury

Martha Calalang[1], Jingang Li[1], Margie-Castillo Melendez[1], Tamara Yawno[1], Courtney McDonald[1], Graham Jenkin[1,2,3], Suzie Miller[1,2,3]

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Background: Preterm birth (<37 weeks of gestation) constitutes 10-12% of all live births in established countries, and results in an immature neurovascular structure that predisposes the preterm brain to haemorrhage. This is a major cause of adverse neurodevelopmental outcomes such as Cerebral Palsy in premature infants, and no treatment exists to protect the brain against vascular injury. Umbilical cord blood (UCB), however, may be efficacious in preventing this in the preterm brain following a hypoxic challenge.

Aims/Hypothesis: This study aims to observe the efficacy of UCB therapy on hypoxia-induced vascular injury and to compare early versus late intervention in a fetal ovine model. We hypothesize that UCB treatment will ameliorate injury and that earlier therapy will be more effective in neurovascular protection.

Methods: Hypoxic injury was induced in fetal sheep via umbilical cord occlusion (UCO) at 102 days gestational age (GA). Term ovine UCB from healthy pregnancies was administered at 12 hours or 5 days post-UCO. Post-mortem was performed at 112 days GA (10 days post UCO) and fetal brains were collected for analysis. Four groups were studied: control (n=7), UCO (n=7), UCO + UCB 12h (n=7), UCO + UCB 5d (n=6) and control + UCB (n=5). The ability of UCBs to facilitate vessel maturation (GLUT-1) and expansion (laminin), as well as blood-brain barrier (BBB) permeability (albumin) was examined using immunohistochemistry.

Results: GLUT-1 immunohistochemistry showed a significant increase in control vessel numbers compared to UCO in the subventricular zone (SVZ), periventricular (PVWM) and cortical (CWM) white matter ($p<0.05$). Early treatment yielded more vessels in the PVWM versus delayed ($p=0.019$). The diameter ($p=0.0303$) and length ($p<0.0001$) of blood vessels increased in both UCO animals compared to controls, and in early therapy versus delayed ($p<0.05$). Laminin staining displayed a trend of improved morphology in both control versus UCO, as well as early treatment compared to delayed, though these results were not significant. The albumin stain presented extravasation across all groups, but no significant difference in severity was observed.

Conclusions: Early UCB treatment was found to be more effective than delayed therapy in expanding the cerebral vasculature of the preterm brain following hypoxia. Increased BBB permeability was seen in both the control and UCO preterm brain, with UCB therapy having no effect in protecting the BBB.

Systemic flow increases after ductal closure are related to redistribution of arterial reservoir discharge in preterm newborn lambs

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Background: Recent findings indicate that ~2/3 of left-to-right shunting across the ductus arteriosus in preterm newborn lambs arises via diastolic discharge from two arterial reservoirs linked via the aortic isthmus, namely 1) an upper body reservoir in the ascending aorta and major cephalic arteries, and 2) a lower body reservoir in the descending thoracic/abdominal aorta and its major branches. This implies that changes in major regional systemic blood flows occurring with ductal closure are related to a redistribution of these reservoir discharges.

Aims: To investigate changes in upper and lower body arterial reservoir (i.e. diastolic) discharges occurring with ductal closure and quantify their contribution to associated alterations in major regional arterial blood flows.

Methods: Anaesthetized fetal lambs were instrumented at 128±1 days with 1) carotid arterial (CA), brachiocephalic trunk (BCT), aortic isthmus (AI) and ductal flow probes, 2) aortic (AoT) and pulmonary trunk (PT) catheters. After caesarean section delivery and mechanical ventilation for 2 hrs, the ductus was closed with a snare while haemodynamics were recorded. During analysis, mean, systolic and diastolic blood flows were obtained after flow in the descending thoracic aortic (DTA) was calculated as the sum of AI and ductal flows.

Results: With ductal closure 1) AoT pressures rose ($P<0.001$) but PT pressures fell ($P<0.001$), 2) mean CA flow rose 12% ($P<0.001$), with a 7% fall in systolic flow ($P<0.002$) but a 73% rise in diastolic flow ($P<0.001$), 3) mean BCT flow rose 16% ($P<0.001$), with a 13% fall in systolic flow ($P<0.001$) offset by a negative-to-positive switch in diastolic flow ($P<0.001$), 4) mean AI flow fell 28% ($P<0.001$), due to a 12% fall in systolic flow ($P<0.05$) and a positive-to-negative switch in diastolic flow ($P<0.001$), and 5) mean DTA flow rose 20% ($P<0.02$), with a 24% fall in systolic flow ($P<0.001$) offset by an 82% reduction in negative (i.e. retrograde) diastolic flow ($P<0.001$).

Conclusions: Increased systemic arterial flows after ductal closure are primarily due to a redistribution of arterial reservoir discharge, with a rise in cephalic flows supported by reversal of upper body reservoir discharge across the AI, and a rise in DTA flow supported by a marked fall in retrograde discharge from the lower body reservoir.

The effect of postnatal steroids on heart structure in preterm lambs

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Background: Advances in the postnatal clinical care of preterm infants have improved survival rates, but at what cost? The administration of postnatal steroids in neonates with severe bronchopulmonary dysplasia improves respiratory function but has known side effects; one adverse consequence is the induction of transient hypertrophic cardiomyopathy. As such, the delayed effects of postnatal steroid treatment on the heart warrants investigation.

Aims/Hypothesis: To determine the effects of postnatal dexamethasone therapy on the structure of the heart in lambs delivered preterm.

Methods: Female lambs were delivered preterm at 128 d GA (term ~150 d GA) and managed postnatally in the UWA Preclinical Intensive Care Research Unit (PICRU) following contemporary clinical practice. From postnatal days 3-12, treated lambs (n=6) were administered tapered doses of dexamethasone (0.15 mg/kg/d (3 d); 0.10 mg/kg/d (2 d); 0.05 mg/kg/d (2 d) and 0.02 mg/kg/d (2 d)), and controls (n=6) received an equivalent volume of saline. Lambs were euthanised at 2 months of age, and the hearts were weighed and perfusion-fixed. Ventricular wall thickness was measured, then heart tissue was randomly and systematically sampled for assessment of myocardial collagen content and cardiomyocyte nuclei number.

Results: Postnatal steroid treatment did not affect body weight, heart weight or ventricular wall thickness in female preterm lambs. The percentage of myocardial collagen was not significantly different between groups. Analyses of the number of cardiomyocytes is currently under investigation.

Conclusions: Postnatal steroid treatment does not overtly influence cardiac morphology in female lambs born preterm. Future analyses will highlight any cardiac cellular changes in response to steroid treatment.

Dexamethasone increases the stiffness of the developing lamb aorta

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Background: Mothers at risk of preterm delivery frequently receive treatment with glucocorticoids to induce fetal lung maturation. Glucocorticoid treatment reduces aortic stiffness in the presence of systemic inflammatory diseases. However, the impact of glucocorticoids on the biomechanics of the developing aorta is poorly understood. Changes in arterial biomechanics can lead to early-onset atherosclerosis and arterial disease. Characterising how glucocorticoids alter biomechanics may provide crucial insight into early life onset of cardiovascular disease.

Aims/Hypothesis: We tested the hypothesis that dexamethasone would increase the stiffness and ultimate tensile strength of the distal aorta in preterm lambs.

Methods: Preterm lambs were delivered at 129 d gestation and randomised to a reducing postnatal dexamethasone course (commencing at 0.15 mg/kg/d), or an equivalent volume of saline. Lambs were maintained for either 7 d (saline: n=3, dex: n=6) or 2 m (saline: n=4, dex: n=4), prior to euthanasia. Aortas were excised and cut into ring specimens, and subjected to uniaxial tension experiments until failure. Displacement, force and vessel dimensions data were used to obtain stress and strain values, which we used for the calibration of a first-order Ogden-type strain energy function for direct comparison.

Results: Postnatal dexamethasone did not affect aortic stiffness at 7 d ($p=0.08$). However, lambs exposed to dexamethasone showed a markedly increased stiffness in distal aortic segments at 2 m, indicated by an increased α parameter ($p=0.02$). There was no significant effect of dexamethasone on failure force ($p=0.06$). However, failure force was greater in 2 m lambs than 7 d lambs ($p<0.01$).

Conclusion: Dexamethasone significantly increases the stiffness of the distal aorta after 2 months in preterm lambs. As increased stiffness is an independent predictor of cardiovascular disease, postnatal dexamethasone may increase propensity to development of cardiovascular disease later in life.

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Session 5

Chairs – Rosemary Horne and Leo Leader			
10.00am	**A17**	**Samantha Rodrigues (E)**	**(Pro)renin receptor knockdown reduces rate of cell proliferation in a first trimester extravillous trophoblast cell line, HTR-8/SVneo**
10.15am	**A18**	**Anya Arthurs (E)**	**Oxygen regulation of placental miRNA expression**
10.30am	**A19**	**Saije Morosin (E)**	**The role of the prorenin receptor ((P)RR) and soluble prorenin receptor (s(P)RR) in syncytialisation**
10.45am	**A20**	**Nadia Bellofiore (L)**	**Behavioural changes in the spiny mouse are indicative of pre-menstrual syndrome**
11.00am	**General discussion**		

(Pro)renin receptor knockdown reduces rate of cell proliferation in a first trimester extravillous trophoblast cell line, HTR-8/SVneo

Samantha L. Rodrigues[1,2], Riazuddin Mohammed[1,2], Sarah J. Delforce[1,2], Eugenie R. Lumbers[1,2] Kirsty G. Pringle[1,2]

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Background: Successful placentation is critical for healthy fetal development. Placental development requires trophoblast cells to proliferate and invade maternal uterine tissues. The placental renin-angiotensin system (RAS) has been implicated in placentation, with high levels of RAS components found in first trimester placentae. Binding of inactive prorenin to the (pro)renin receptor ((P)RR) can initiate the generation of angiotensin II (Ang II), the main effector peptide of the RAS pathway. Ang II can then bind to the angiotensin type 1 receptor (AT_1R) to stimulate angiogenesis, and proliferation and invasion by trophoblasts. The binding of prorenin to (P)RR can also stimulate intracellular signalling in its own right. However, the functional interaction of prorenin/(P)RR has yet to be characterised in placental development.

Aims/Hypothesis: We hypothesise that prorenin interacting with (P)RR is a key regulator of placental morphogenesis.

Methods: The first trimester extravillous trophoblast cell line HTR-8/SVneo was transfected with Stealth siRNA targeting the *ATP6AP2* gene to knockdown (P)RR expression. q-PCR was performed to determine efficacy of knockdown and its effects on proliferation were measured in real time using the xCELLigence Real-Time Cell Analysis system.

Results: q-PCR analysis showed a 90% reduction in ATP6AP2 mRNA expression ($P < 0.0001$) after (P)RR siRNA transfection when compared with non-transfected control. Real-time monitoring of cell proliferation showed an approximate 30% decrease in proliferation rates of (P)RR siRNA transfected cells when compared with negative control siRNA treated cells ($P < 0.0001$).

Conclusions: Results indicate that (P)RR plays a significant role in trophoblast proliferation in the early placenta. Further experiments are needed to elucidate its role in trophoblast invasion and angiogenesis.

Oxygen regulation of placental miRNA expression

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Background: The renin-angiotensin system (RAS) plays an important role in placentation and components of the RAS are localised within the placenta. Early development occurs in a hypoxic environment in the first trimester; a time when placental RAS expression is highest. We have previously shown that miRNAs may play a role in post-transcriptional regulation of RAS components, as we observed a negative correlation between miRNA expression and RAS mRNA expression with gestational age. It is possible that oxygen levels within the placenta regulate the expression of some of these miRNAs that target the RAS.

Aims/Hypothesis: To demonstrate the effects of oxygen on miRNA expression in first trimester trophoblasts.

Methods: HTR-8/SVneo cells were cultured in 1%, 5% and 20% oxygen. Total RNA was extracted and underwent Affymetrix miRNA microarray analysis (Ramaciotti, UNSW). Results were analysed using Partek Genomic Suite Software. Differences in miRNA expression profiles between oxygen tensions were validated by quantitative RT-PCR (TaqMan). The effect of oxygen on expression of RAS genes and proteins is also being assessed.

Results: The responses of 16 miRNA genes targeting RAS components, in response to oxygen, were assessed. Of these, 12 were shown to be significantly altered in 1% oxygen tension (compared with 5-20%) in the Affymetrix miRNA microarray, and 11 of these were validated using qPCR. Of these 11 miRNAs, 10 had decreased expression in 1% oxygen. These results are consistent with our previous study, which showed that in the first trimester of pregnancy miRNAs targeting the RAS are down-regulated, when placental RAS function is highest. This experiment shows that oxygen does regulate placental miRNA expression.

Conclusions: Oxygen tension and miRNAs interact to regulate the placental RAS in the first trimester of pregnancy. We propose that the premature onset of maternal blood flow to the placenta results in increased oxygen, which in turn increases miRNA expression, decreases RAS activity and ultimately limits placentation.

The role of the prorenin receptor ((P)RR) and soluble prorenin receptor (s(P)RR) in syncytialisation

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Background: The placental syncytiotrophoblast regulates nutrient and waste exchange between the fetus and the mother. Syncytialisation involves placental cytotrophoblast cells continuously fusing to the outer syncytial layer. Insufficient syncytialisation has been associated with preeclampsia and intrauterine growth restriction. The placental renin angiotensin system (RAS) is important for appropriate placental development. Reliant on the renin precursor, prorenin, binding to the prorenin receptor ((P)RR) to initiate the classical RAS cascade, prorenin/(P)RR binding can also induce intracellular signalling pathways important in placental development. A novel form of the (P)RR, the soluble (P)RR (s(P)RR) has been discovered, which in human glomerular epithelial cells, is cleaved by the pro-protein convertase enzyme furin.

Aims/Hypothesis: We hypothesised that the (P)RR is essential for syncytialisation and that syncytialisation will increase the expression and/or secretion of (P)RR, s(P)RR and furin in BeWo choriocarcinoma cells. Furthermore, we postulated that furin cleaves the (P)RR to release s(P)RR from the placenta.

Methods: BeWo choriocarcinoma cells were treated with either forskolin (100µm) or Vehicle (DMSO) control to induce syncytialisation. Additionally, BeWo cells were transfected overnight with either a (P)RR siRNA or furin siRNA, prior to treatment with forskolin. Cells and supernatant were collected after 48 hours incubation and underwent PCR, ELISA and immunocytochemistry analyses. Syncytialisation was quantified by measuring hCG secretion and E-cadherin expression.

Results: Syncytialisation significantly increased s(P)RR secretion into the supernatant ($P=0.0006$). A significant decrease in syncytialisation of the BeWo cells was found upon treatment with (P)RR siRNA ($P=0.004$). Furin knockdown successfully decreased s(P)RR secretion into the supernatant ($P\leq0.001$).

Conclusions: Both the (P)RR and s(P)RR have roles in syncytialisation. Furthermore, furin is responsible, to some extent, for the cleavage of the s(P)RR in BeWo cells.

Behavioural changes in the spiny mouse are indicative of pre-menstrual syndrome

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Background: Pre-menstrual syndrome (PMS) is a prominent disorder that affects millions of women worldwide. Up to 90% of women endure symptoms of impending menstruation, such as bloating, abdominal cramping and nausea. In addition to these physiological aggravations, emotional stressors including depression and anxiety frequently occur in unison. Consequently, over 75% of women experience recurrent symptoms extreme enough to disrupt day-to-day life, and seek intervention. Due to a lack of an appropriate animal model, the mechanisms of PMS are poorly understood, and subsequently, effective treatments are limited.

Aims/Hypothesis: Our laboratory has discovered the first menstruating rodent, the spiny mouse (*Acomys cahirinus*). We now seek to determine whether spiny mice exhibit behavioural changes in correlation with stages of the menstrual cycle. We hypothesise that spiny mice experience an analogous pre-menstrual state.

Methods: We performed vaginal lavages on n=14 virgin female spiny mice (6-8 months of age) and recorded qualitative behavioural responses to researcher handling on an increasing scale of severity (e.g. restraint, frequency of vocalisations, total handling time). The early follicular phase of their cycle was used as the within subject control. The late luteal and early menstrual phases were designated pre-menstrual phases. Ovariaectomised age-matched females (n=5) were used as experimental controls. Generalised Estimating Equations were used to calculate odds ratios.

Results: Several parameters were significantly correlated with the pre-menstrual phases of the cycle, the most prominent being cycling females were 8.4 times more likely to vocalise, and 1.4 times more likely to vocalise in general than controls. We saw significant increases in handling time and overall stress during the pre-menstrual phases, and also compared to ovariacetomised controls.

Conclusions: Spiny mice show behaviours indicative of increased anxiety during pre-menstrual phases. Quantification with validated rodent behavioural tests is ongoing, with expected confirmation of pre-menstrual symptoms.

Session 6

Chairs – Alistair Gunn and Jane Pillow			
11.45am	**A21**	**Jonathan Davis (ECR)**	**Postnatal dexamethasone effect on preterm lamb lung development**
12.00pm	**A22**	**Erin McGillick (ECR)**	**Elevated airway liquid volumes at birth: A potential cause of transient tachypnea of the newborn**
12.15pm	**A23**	**Kyra Chan (E)**	**Isolating effects of the initiation of ventilation on the preterm lamb brain**
12.30pm	**A24**	**Shigeo Yamaoka**	**Understanding the effect of transient tachypnea of the newborn on cardiorespiratory function in premature newborn lambs**
12.45pm	**General discussion**		

Postnatal dexamethasone effect on preterm lamb lung development

Jonathan W. Davis[1], Peter Noble[2], Siavash Ahmadi-Noorbakhsh[2], Amy Wooldridge[2], M.J. Dahl[3], Kurt Albertine[3], Paris Papagianis[4], Jane J. Pillow[1,2]

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Background: Postnatal dexamethasone (DEX) is effective therapy for preterm infants with severe bronchopulmonary dysplasia and to wean mechanical ventilation. DEX treatment is controversial due to neurodevelopment concerns. Current DEX treatment targets lower doses and rapid weaning. The effects on organ development are unknown.

Aims: To compare the effects of low- and high-dose DEX on alveolar architecture in preterm lambs.

Methods: Date-mated ewes received betamethasone at 48h and 24h prior to delivery at 128-130d gestation. Lambs were cannulated, intubated and administered surfactant prior to delivery, resuscitated and received volume-targeted ventilation. Lambs had graded reduction of respiratory support. Postnatal lambs were randomly assigned to: 1) saline 2) low-dose DEX (LD-DEX; total 0.75mg/kg) or 3) high-dose DEX (HDDEX; total 1.62 mg/kg) commencing < 2 h of birth. Lambs were euthanised on day 7. A fetal control group (135-137d; FC) were exteriorised and euthanised immediately. Three sections of lung and five regions per section were selected per lamb for paraffin embedding and unbiased stereology. Airspace/tissue fractions and secondary septal crest (SSC) density were calculated. Analysis was by 1-way ANOVA and Student–Newman–Keuls

Results: Lung airspace/tissue fraction did not differ between groups. SSC density was different across the groups (P=0.036). Septal crest density was reduced in each group compared to maturation-matched controls (P<0.05, post-hoc analysis). Postnatal DEX had no effect on SSC density or other outcomes.

	Saline	LD-DEX	HD-DEX	FC
Female/Male	5/6	2/5	3/5	3/2
Gestation (d)	129.7 (1.3)*	128.1 (0.9)*	128.3 (0.5)*	136.6 (1.1)
Postnatal age (d)	7.4 (1.6)*	6.9 (0.6)*	8.0 (0.9)*	0 (0)
PM Weight (kg)	2.8 (0.4)*	2.7 (0.3)*	2.7 (0.4)*	3.9 (0.5)
Airspace Fraction	0.53 (0.06)	0.50 (0.07)	0.48 (0.10)	0.44 (0.07)
Tissue Fraction	0.44 (0.06)	0.47 (0.07)	0.49 (0.10)	0.50 (0.05)
Septal Crest Density	0.081 (0.018)*	0.075 (0.027)*	0.071 (0.022)*	0.11 (0.030)

Data are mean (SD). PM - post mortem; LD-DEX- low dose dexamethasone; HD-DEX- high dose dexamethasone; FC fetal control. * p<0.05 compared to fetal control

Conclusions: Secondary septation is reduced in 7d old preterm lambs compared to maturation-matched controls, despite mechanical ventilation. Postnatal DEX does not prevent nor exacerbate this alveolarisation.

Elevated airway liquid volumes at birth: A potential cause of transient tachypnea of the newborn

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Background: The rate of preterm birth and non-labour caesarean delivery has substantially increased, which delays lung liquid clearance and increases the risk of neonatal respiratory complications. Retention of liquid in airways and/or lung tissue is thought to underpin the respiratory morbidity associated with transient tachypnea of the newborn (TTN). However, its effects on respiratory structure and function after birth is unknown.

Aim: To investigate the effect of elevated airway liquid volumes on lung aeration and respiratory function in the immediate newborn period.

Methods: Preterm rabbit kittens were delivered at 30d gestation (term=32d). Kittens had lung liquid drained (Control, n=7) or liquid added to the lungs to mimic TTN (30mL/kg saline, LA, n=7). Kittens were mechanically ventilated to assess respiratory mechanics. Synchrotron phase contrast X-ray images were obtained and analysed to determine regional lung gas volumes, airway dimensions, chest and lung measurements.

Results: The LA kittens required a higher maximum peak inspiratory pressure than Controls to recruit a tidal volume of 8 mL/kg. LA kittens required greater time to achieve lung aeration and exhibited regional differences in lung aeration and airway size. LA kittens had increased thoracic expansion as evidenced by increased diaphragm curvature, greater total chest area and increased lung height. During the ventilation sequence, decreased positive end expiratory pressure (distending pressure applied to the lung at end expiration) resulted in reduced functional residual capacity in the LA compared to Control kittens. This indicates liquid re-entry into the airways and provides evidence for decreased respiratory function in LA kittens.

Conclusions: Elevated lung liquid volumes in the LA kittens had marked adverse effects on lung structure and function in the immediate neonatal period. This reduced the ability of the lung to aerate efficiently and maybe the mechanism underlying TTN in near term babies after birth.

Isolating effects of the initiation of ventilation on the preterm lamb brain

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Background: Initiation of ventilation in the delivery room increases the risk and severity of brain injury in preterm neonates through two major pathways: by initiating an inflammatory cascade and causing cardiovascular and cerebral haemodynamic instability. However, the relative contribution of each pathway on preterm brain injury is not known.

Aims/Hypothesis: To characterise the relative contributions of the two major pathways of ventilation-induced cerebral white matter injury in preterm lambs.

Methods: Fetal lambs (0.85 gestation) were exteriorised, ventilated with an injurious ventilation strategy for 15 min either with placental circulation intact to maintain stable haemodynamics (INJ_{INF}; n=5) or umbilical cord occluded ($INJ_{INF+HAE}$; n=7), then returned to the uterus. Sham controls were exteriorised but not ventilated (SHAM; n=5) while unoperated controls (UNOP; n=6) did not undergo sterile surgery. At 24 h, lambs were delivered and maintained on non-injurious ventilation for up to 1 h during which lambs underwent magnetic resonance spectroscopy (MRS) and diffusion tensor imaging (DTI) using a 3T MRI scanner. Immunohistochemistry and qRT-PCR were used to assess inflammation, cell death, and vascular leakage. One-way ANOVA was used for statistical analysis. Values of $p<0.05$ were deemed statistically significant.

Results: At 24 h, MRS and DTI detected significant differences between INJ lambs and controls within the internal capsule and frontal white matter. Histological confirmation of injury is currently being undertaken to confirm the detected injury corresponds to pathological injury.

Conclusions: Initial investigations suggest that injurious ventilation, irrespective of the strategy, increases brain inflammation and injury compared to controls. A thorough understanding of the pathways of ventilation-induced brain injury can aid development of targeted treatments to reduce VIBI and improve neurological outcomes of preterm babies.

Understanding the effect of transient tachypnea of the newborn on cardiorespiratory function in premature newborn lambs

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Background: Transient tachypnea of the newborn (TTN) is a respiratory complication that is thought to result from delayed clearance of fetal lung liquid following birth. However, the precise mechanisms regulating this and the effects of elevated airway liquid volumes on the fetal to neonatal cardiorespiratory transition at birth is unknown. We have developed a sheep model mimicking elevated airway liquid volumes associated with TTN to investigate real-time changes in cardiopulmonary and respiratory function in premature newborns.

Aims/Hypothesis: To investigate the effect of elevated airway liquid volume associated with TTN on cardiorespiratory function in preterm newborn lambs.

Methods: Preterm lambs (130d gestation, term ~147d) were instrumented to comprehensively investigate blood pressure, blood flow, oxygenation and intra-thoracic pressure in real-time during the fetal to neonatal transition. Immediately before birth, lung liquid was drained. Lambs were randomly assigned to receive either 30mL/kg of Hartman's solution (Liquid added (LA) to mimic TTN, n=6) or no liquid replaced (Control, n=6). After umbilical cord clamping, the lambs were mechanically ventilated for 1 hour and regular blood gas samples were taken. During ventilation, the fraction of inspired oxygen (FiO_2) and/or ventilator settings were manually adjusted to maintain oxygen saturation and carbon dioxide concentration within a physiological target range in both groups. At the conclusion of the experiment wet and dry lung weight was determined. For all physiological recordings the effect of treatment (Control vs. LA) and time after birth was investigated.

Results: Compared to Control lambs, LA lambs had persistent hypercapnia and acidosis despite requiring greater supplemental oxygen (higher FiO_2). The LA lambs had reduced gas exchange capabilities evidenced by reduced oxygenation index and alveolar arterial difference in oxygen. The LA group had higher lung wet/dry weight ratio indicating liquid accumulation in lung tissue. There was no effect on blood flow or blood pressure.

Conclusions: In this model, we provide evidence for adverse effects of excess airway liquid volume on respiratory but not cardiovascular function in preterm newborn lambs. Future studies using this model will be conducted to compare effects of elevated lung liquid volumes on cardiopulmonary function in near-term lambs.

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Session 7

Chairs – Tamás Zakár and Hayley Dickinson			
2.00pm	A25	Paris Papagianis (L)	Viability and function of human amnion epithelial cells is not altered by temperature
2.15pm	A26	Tayla Penny (E)	Does administration of umbilical cord blood cells ameliorate long term behavioural effects following neonatal hypoxic ischemic brain injury?
2.30pm	A27	Laura Bennet	Delayed intranasal infusion of human amnion epithelial cells improves white matter maturation after asphyxia in preterm fetal sheep
2.45pm	A28	Madison Paton (L)	Umbilical cord blood therapy modulates LPS-induced brain injury in preterm fetal sheep
3.00pm	General discussion		

Viability and function of human amnion epithelial cells is not altered by temperature

Paris Papagianis[1,2,3], Courtney McDonald[1,2], Jane Pillow[3], Tim Moss[1,2]

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Background: Human amnion epithelial cells (hAECs) are immunomodulatory, and a promising cell therapy for neonatal disease. The effect of temperature on hAEC viability and function is unknown, but may influence the efficacy of hAECs in experimental animals with different body temperatures (e.g. sheep ~39°C; mice ~36°C), patients with fever (>38°C) or in infants undergoing therapeutic hypothermia (~33°C). We aimed to determine whether viability and function of hAECs is altered when cultured at 33°C, 37°C or 39°C.

Methods: hAECs were seeded at 500,000 cells/well in 6-well plates and incubated at 33°C, 37°C or 39°C for 24h, 48h or 72h. Viability was assessed by flow cytometry. Function was assessed using a wound healing assay: cells were cultured at 37°C until confluent, and then scratched and incubated at 33°C, 37°C or 39°C. Images were taken at 0h and 72h to compare wound closure. Phagocytosis assays were performed using immortalised mouse macrophages (iMACs): iMACs were seeded at 50,000 cells/well in a 96-well plate and incubated with or without hAEC-conditioned media (33°C, 37°C or 39°C) and fluorescent beads for 3h at 37°C. Uptake of fluorescent beads by iMACs was measured by flow cytometry.

Results: Viability of hAECs was not different at 33°C, 37°C or 39°C, although viability declined over 72h ($p<0.05$) at all temperatures. Wound closure was not different between hAECs incubated at 33°C, 37°C or 39°C. Phagocytic function of iMACs was not different when treated with conditioned media from hAECs incubated at 33°C, 37°C or 39°C.

Conclusions: Viability and function of hAECs *in vitro* is not altered by incubation temperatures between 33-39°C. Therefore, hAECs are likely to be of equal therapeutic effect in patients across a realistic range of body temperatures

Does administration of umbilical cord blood cells ameliorate long term behavioural effects following neonatal hypoxic ischemic brain injury?

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Background: Severe hypoxic ischemic (HI) injury at birth is the most readily identifiable cause of long term neurodevelopmental disorders, such as cerebral palsy (CP). This study explored the potential of human umbilical cord blood (hUCB) cells as a treatment for HI brain injury.

Aims/Hypothesis: The aim of this project was to assess the behavioural and physiological changes in a neonatal HI rat model following administration of hUCB cells. It was hypothesised that hUCB cells would reduce HI brain injury, and show long-term behavioural improvements.

Methods: HI injury was induced in post-natal day (PND) 7 rats by ligation of the left carotid artery, followed by exposure to hypoxia (8%O_2) for either 1.5 or 3 hours. 24 hours post-HI, rats received either whole hUCB mononuclear cells (MNCs), or specific cell types found in hUCB; monocytes, endothelial progenitor cells (EPCs) or regulatory Tcells (Tregs). Rats were monitored until PND50, throughout this period they underwent negative geotaxis analysis, open field testing, novel object recognition and forelimb preference analysis. On PND50, rats were culled and brains collected for immunohistological analysis.

Results: HI injury resulted in large infarcts in the left hemisphere, and increased activation of microglia following cell treatment, which is predicted to be a "reparative" M2 microglial population. Significant injury to the motor cortex of the left hemisphere resulted in impairment in forelimb use and a significant preference to the uninjured side was found in HI injured rats. Treatment with whole UCB mononuclear cells, Tregs and Monocytes, following a 1.5hr hypoxic injury, showed evidence of reducing injury to the motor cortex, indicated by a reduction in left forelimb asymmetry.

Conclusions: This study has shown that following a HI injury, delivery of hUCB cells ameliorated injury in the motor cortex, and potentially caused polarisation of M2 microglia at the site of injury.

Delayed intranasal infusion of human amnion epithelial cells improves white matter maturation after asphyxia in preterm fetal sheep

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Background: Perinatal hypoxic-ischemic brain injury remains highly associated with cerebral palsy after preterm birth. The timing of injury is often unclear, and thus it is critical to identify interventions that are effective despite significant delay after injury. Preterm infants are vulnerable to white matter injury, in modern human cohorts long-term recovery is associated with effective restoration of total numbers of oligodendrocytes, but impaired cell maturation leading to impaired myelination, and long-term disability.

Aims/Hypothesis: To evaluate whether delayed treatment with human amnion epithelial cells (hAECs) started 24 hours after severe hypoxia-ischemia in preterm fetal sheep can improve long-term maturation of white matter.

Methods: hAECs were infused intranasally at 1, 3 and 10 days after severe asphyxia induced by umbilical cord occlusion for 25 minutes. Histological outcome was assessed 3 weeks after asphyxia.

Results: After 21 days recovery, asphyxia was associated with impaired maturation of oligodendrocytes (OLs), with no significant loss of total OLs but a marked reduction in immature/mature OLs and reduced myelination of the white matter tracts. Small numbers of hAECs were present in the brain parenchyma 3 weeks after intranasal administration. The proportion of immature/mature OLs and myelination were markedly improved, to sham control values, with reduced numbers of active microglia and numbers and area of astrogliosis.

Conclusions: Delayed intranasal infusion of hAEC was associated with improved neuronal survival in deep grey matter, and reduced apoptosis and TNFα staining in the cortex. These findings suggest that delayed intranasal hAEC administration has potential to improve long term recovery from brain injury after perinatal HI.

Umbilical cord blood therapy modulates LPS-induced brain injury in preterm fetal sheep

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Background: Up to 70% of all preterm births have been complicated by inflammation of the placenta or its membranes, termed chorioamnionitis. Chorioamnionitis during development can significantly increase brain injury and the risk of cerebral palsy, thus there is a need for new therapies. This study models preterm white matter injury and explores the therapeutic potential of stem cells isolated from human umbilical cord blood (UCB).

Aims/Methods: This study aims to elucidate the therapeutic potential of UCB stem cells for preterm brain inflammation. It is hypothesised that stem cells will attenuate the brain injury associated with chorioamnionitis.

Methods: White matter injury was induced in fetal sheep at 0.65 gestation. LPS (150ng I.V., 055:B5), or saline for controls, was administered directly to the fetus over 3 consecutive days. Stem cell-treated animals received 100 million UCB mononuclear cells (I.V.), 6 hours after the final LPS dose. Brains were collected for analysis at 10 days after the final LPS dose.

Results: LPS administration increased microglial activation (Iba-1) and cell death (Cas-3, $p<0.05$) in the subcortical white matter and cortex, compared to controls. UCB attenuated this injury. LPS administration also resulted in increased spleen and adrenal weights, indicating a systemic immune/stress response.

Conclusions: UCB treatment reduced white matter injury following LPS-induced brain injury. Future studies will examine if cord tissue stem cells or repeated doses provide even greater benefit.

Session 8

Chairs – Tim Moss and Kirsty Pringle			
3.45pm	**A29**	**Jago Van Dam (E)**	**Reduced cortical excitability, neuroplasticity and cortisol in children exposed to gestational diabetes in utero: Is metformin neuroprotective**
4.00pm	**A30**	**Nathanael Yates (ECR)**	**Developmental Vitamin D deficiency and autism spectrum disorder phenotypes in male rats**
4.15pm	**A31**	**Kelsee Shepherd (E)**	**Cardiorespiratory events in preterm infants: Effect of sleeping position, sleep state and postnatal age**
4:30pm	**General discussion**		

Reduced cortical excitability and neuroplasticity in children exposed to gestational diabetes in utero

Jago Van Dam[1], Luke Schneider[1], Amy Garrett[1], Mitchell Goldsworthy[1], Nicolette Hodyl[1], Bill Hague[2], Julia Pitcher[1]

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Background: Children exposed to gestational diabetes mellitus (GDM) in utero are at increased risk of suboptimal neurodevelopment, including attention deficits. Metformin offers an alternative to insulin therapy for GDM but, unlike insulin, crosses the placenta and directly interacts with the fetus raising safety concerns. However, metformin is pro-neurogenic and anti-inflammatory and may provide some fetal neuroprotection.

Aims/Hypothesis: The main aim of this study was to investigate cortical excitability and the response to long-term depression (LTD)-like neuroplasticity induction in 11 – 13-year-old children exposed to GDM in utero.

Methods: We used non-invasive brain stimulation techniques to investigate cortical excitability and neuroplasticity in 27 GDM-exposed and 11 matched non-GDM-exposed control children, aged 11-13 years. The GDM children were born to participants in the MiG RCT of metformin versus insulin to treat GDM. Saliva was collected immediately prior to brain stimulation for later cortisol analysis.

Results: GDM children had reduced cortical excitability ($F_{(2,32)}$ = 3.39, P = 0.046) compared with controls, and reliable motor potentials could not be evoked in 7 GDM children. Compared with controls, GDM-exposed children had a reduced or absent neuroplastic response ($F_{(1,28)}$ = 6.76, P = 0.015) to brain stimulation, and this was associated with lower cortisol levels than their unexposed peers (P = 0.001), and higher maternal insulin resistance at trial entry (P = 0.023). However, metformin-exposed children exhibited more normal neuroplastic responses than insulin-exposed children, despite similar cortisol levels.

Conclusions: Metformin might offer some neuroprotection to GDM-exposed fetuses, but does not prevent HPA axis programming i.e. low cortisol. The findings regarding maternal insulin resistance at trial entry suggest that we may not be treating women for GDM early enough in pregnancy to prevent adverse neurological development in the fetus.

Developmental Vitamin D deficiency and autism spectrum disorder phenotypes in male rats

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Background: Maternal vitamin D deficiency is common, and is suggested to play a role in autism spectrum disorder (ASD). However, the relationship between developmental vitamin D deficiency and ASD traits such as poor social, communication and language development, has not been determined experimentally.

Aims/Hypothesis: We hypothesised that maternal vitamin D deficiency would contribute to neurodevelopmental traits consistent with ASD, in a rodent model.

Methods: Female Sprague-Dawley rats were provided with vitamin D deficient (0 IU/kg, N = 15) or vitamin D control (2,195 IU/kg, N = 15) diets for a minimum of 5 weeks before mating, and maintained until offspring weaning. On PND1 pup litter size was standardized (N = 5 males/litter), and neurodevelopmental markers were assessed from PND3 – PND14. Social communication was assessed on PND12 by recording ultrasonic vocalisations of pups repeated maternal separation. Between 3 – 4 months all animals went through a memory tests, a social interaction test, MRI scans, and brain histology.

Results: Vitamin D deficiency had no effect on litter characteristics, offspring weight, or neurodevelopmental milestones. Repeated maternal separation in control animals increased ultrasonic vocalisations (2.7 – fold change, χ^2=6.89, df = 1, p<0.01). This was not the case for vitamin D deficient animals (1.04 – fold change, χ^2=0.05, df = 1, p > 0.05) which also had decreased cortical levels of the language associated protein FOXP2. Adult vitamin D deficient animals demonstrated subtle decreases in recognition memory (object-in-place, t = 2.461, df = 16, p < 0.05), reduced social interaction (t = 2.509, df = 24, p < 0.05), and no changes in indicators of anxiety or depression. Vitamin D deficient rats exhibited a 2.4-fold increase in lateral ventricle volume compared to controls (MRI, p < 0.05), but no changes in the hippocampus, corpus callosum or cerebellum.

Conclusions: Maternal vitamin D deficiency affected in male rodents broadly consistent with an ASD-like. This supports epidemiological evidence that vitamin D deficiency influences offspring neurodevelopment.

Cardiorespiratory events in preterm infants: Effect of sleeping position, sleep state and postnatal age

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Background: Current American Academy of Pediatrics recommendations suggest healthy preterm infants in the neonatal intensive care unit (NICU) should be slept supine by 32 weeks of gestation. However, placing a preterm infant in the prone position, the opposite position recommended for term infants, is frequently employed to improve respiratory function in NICU. To date, no longitudinal data are available on the effect of sleeping position on clinically significant episodes of bradycardia, apnoea and desaturation in preterm infants in their early postnatal days in NICU.

Aims/Hypothesis: To investigate whether sleeping position has any effect on the frequency, duration and severity of bradycardia, apnoea and desaturations in preterm infants over the first 3 weeks of life.

Methods: Twenty three preterm infants (born at 25-33 weeks of gestation) were studied weekly for 3 weeks after birth, for 1hr in both the prone and supine positions. Using routine cardiorespiratory monitoring and oximetry (SaO_2), episodes of bradycardia (heart rate ≤100bpm), apnoea (respiratory pauses ≥10 s) and desaturations (SaO_2 ≤80%) were recorded and analysed. Active sleep (AS) or quiet sleep (QS) states were scored at bedside with established behavioural criteria. Two-way RM ANOVA, with Tukey's multiple comparison test, assessed the effect of sleep position/state and postnatal week.

Results: The number of desaturations/hour of sleep was higher in the supine compared to the prone position in AS at week 3 ($P<0.05$). In the supine position, compared to QS, AS was associated with higher number of desaturations/hour of sleep at week 3, higher number of bradycardias/hour of sleep and higher proportion of time having bradycardias at week 1. In AS-supine, the proportion of time spent having bradycardias was higher in week 1 than week 3. Compared to week 1, 18 infants required the same or less respiratory support at week 3. However, the number of desaturations/hour of sleep in AS-supine was higher in week 3 compared to week 1 & 2, with increased duration of desaturations and lower SaO_2 nadir in week 3 compared to week 1 in QS in both positions.

Conclusions: In preterm infants, during the first 3 weeks of life, the supine position is associated with higher frequency of desaturations during AS, and AS was associated with more desaturations and bradycardic events than QS. The increased desaturations with postnatal age may be related to infants being weaned off respiratory support.

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