Fetal and Neonatal Workshop of Australia and New Zealand

32nd Annual meeting

22 – 23 March 2018

Rydges Lakeland Resort

Queenstown

New Zealand

Organising Committee:

Dr Robert De Matteo

Prof Laura Bennet

Prof Alistair Gunn

Dr Joanne Davidson

Dr Guido Wassink

PERINATAL SOCIETY of Australia & New Zealand PSANZ

THE UNIVERSITY OF AUCKLAND Te Whare Wānanga o Tāmaki Makaurau NEW ZEALAND

MEDICAL AND HEALTH SCIENCES SCHOOL OF MEDICAL SCIENCES

MAURICE & PHYLLIS PAYKEL TRUST

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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 PSANZ

THE UNIVERSITY OF AUCKLAND Te Whare Wānanga o Tāmaki Makaurau NEW ZEALAND

MEDICAL AND HEALTH SCIENCES SCHOOL OF MEDICAL SCIENCES

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FNWANZ 2018 Programme Outline

Rydges Lakeland Resort, Queenstown, New Zealand

Thursday 22nd March

8:00am – 9:00am	Registration	Wakatipu Room
9:00am - 10:30am	Session 1	Queenstown Room
10:30am - 11:00am	Morning Tea	Foyer
11:00am - 12:30pm	Session 2	Queenstown Room
12:30pm - 1:30pm	Delegate Lunch	Bazar Restaurant
1:30pm - 3:00pm	Session 3	Queenstown Room
3:00pm - 3:30pm	Afternoon Tea	Foyer
3:30pm – 5:15pm	Session 4	Queenstown Room
7:00pm – 10:00pm	Delegate Dinner	Prime Restaurant

Friday 23rd March

8:00am – 9am	Registration	Wakatipu Room
9:00am – 10:40am	Session 5	Queenstown Room
10:40am – 11:10am	Morning Tea	Foyer
11:10am - 12:35pm	Session 6	Queenstown Room
12:35pm - 1:35pm	Delegate Lunch	Bazar Restaurant
1:35pm - 3:05pm	Session 7	Queenstown Room
3:05pm - 3:35pm	Afternoon Tea	Foyer
3:35pm – 4:00pm	Prize giving/Farewell	Queenstown Room

FNWANZ 2018 Programme

Thursday 22nd March

8:00am – 9:00am Registration (Wakatipu Room)

Session 1 (Queenstown Room)				
Chairs: A/P Michael Stark and Prof Janna Morrison				
Time	**No.**	**Speaker**		**Student/ECR**
9:00am	A01	Deanne H. Hryciw	Role of linoleic acid in placental inflammation and cell viability	Not Applicable
9:15am	A02	Vicki Clifton	Placental glucocorticoid-regulated pathways in a sheep model of maternal allergic asthma	Not Applicable
9:30am	A03	Nadia Bellofiore	Pocket primate? The spiny mouse as a model for menstrual research	Late PhD
9:45am	A04	Harleen Kaur	A potential role for ghrelin as a driver of increased growth hormone during murine pregnancy	Early PhD
10:00am	A05	Kathryn L Gatford	Developing a fluorescent method to measure placental glucose transport in mice	Not Applicable
10:15am – 10:30am General discussion				
10:30am – 11:00am Morning Tea (Foyer)				

Session 2 (Queenstown Room)				
Chairs: Prof Vicki Clifton and Dr Amy Woolridge				
11:00am	A06	Stephanie Miller	Identification and expression of a unique neonatal variant of the GABAA receptor alpha-3 subunit	Early Career
11:15am	A07	Jago van Dam	Potential mechanisms underlying altered LTD-like neuroplastic responses to brain stimulation in children exposed to gestational diabetes in utero	Early PhD
11:30am	A08	Sonja Brennan	The renal parenchyma - Evaluation of a novel ultrasound measurement to assess fetal renal development	Early PhD
11:45am	A09	Janna Morrison	Fetal Cardiac Haemodynamics: Initial Experience using 4D flow MRI	Not applicable
12:00pm	A10	Jeremy Lum	Maternal Methadone Treatment Causes Cognitive Deficits in Adolescent Offspring: A Rodent Study	Late PhD
12:15pm – 12:30pm General discussion				
12:30pm – 1:30pm Delegate Lunch (Bazar Restaurant)				

Session 3 (Queenstown Room)				
Chairs: Dr Kathy Gatford and Dr Guido Wassink				
Time	**No.**	**Speaker**		**Student/ECR**
01:30pm	A11	Amy Wooldridge	Relationship between size at birth and postnatal allergy	Early career
01:45pm	A12	Jack Darby	Maternal undernutrition increases IGF2 signalling molecules and fibrosis in the heart of the late gestation sheep fetus	Late PhD
02:00pm	A13	Ishmael Inocencio	Sildenafil and Dopamine for Cardiovascular support in FGR lambs	Late PhD
02:15pm	A14	Kirat Chand	Neuropathology in the IUGR piglet model	Early career
02:30pm	A15	Julie Wixey	Ibuprofen treatment to reduce inflammation and neuronal injury in the growth restricted neonatal brain	Early Career
02:45pm – 03:00pm General discussion				
3:00pm – 3:30pm Afternoon Tea (Foyer)				

Session 4 (Queenstown Room)				
Chairs: Prof Laura Bennet and Dr Christopher Lear				
03:30pm	A16	Vivian Tran	The effects of preterm birth and intrauterine inflammation on the structure of the aortic wall	Early PhD
03:45pm	A17	Bianca Le	Cardiac ventricular structure at 2 and 5 months after birth in lambs born very preterm and ventilated in the neonatal period	Late PhD
04:00pm	A18	Jia Yin Soo	The interaction between fetal growth, preterm birth and methamphetamine exposure on drug metabolism	Late PhD
04:15pm	A19	Mitchell Lock	Immediate gene response following myocardial infarction in the fetal and adolescent sheep heart	Late PhD
04:30pm	A20	Victoria King	Effect of chronic inflammation on circadian rhythms in the preterm fetus	Masters
04:45pm	A21	Simerdeep Dhillon	Cardiovascular effects of delayed human recombinant erythropoietin treatment after asphyxia in preterm fetal sheep	Late PhD
05:00pm – 5:15pm General discussion				
07:00pm to 10pm Delegate Dinner (Prime Restaurant)				

Friday 23rd March

8:00am – 9:00am Registration (Wakatipu Room)

Session 5 (Queenstown Room)				
Chairs: Prof Rosemary Horne and Dr Rebecca Dyson				
Time	**NR**	**Speaker**		**Student/ECR**
09:00am	A22	Yogavijayan Kandasamy	Evidence of glomerular injury in preterm neonates	Not applicable
09:15am	A23	Dominique Blache	Neurodevelopmental outcomes after postnatal dexamethasone in preterm lambs	Not applicable
09:30am	A24	Donna Rudd	Discovering the faecal microbiome of pre-term neonates at the Townsville Hospital NICU: First hesitant steps and preliminary findings	Not applicable
09:45am	A25	Christopher Lear	Long-term neurophysiological and histological outcomes after preterm fetal hypoxia-ischemia	Early career
10:00am	A26	Guido Wassink	Can treatment with clonidine reduce white matter damage in asphyctic preterm babies?	Early career
10:15am	A27	Veena Kurup	Long-term effects of low dose dexamethasone and ventilation therapies on alveolar development in preterm lambs	Early PhD
10:25am – 10:40am General discussion				
10:40am – 11:10am Morning Tea (Foyer)				

Session 6 (Queenstown Room)				
Chairs: A/P Tim Moss and Dr Joanne Davidson				
11:10am	A28	Madison Paton	Assessing the efficacy of human umbilical cord blood versus mesenchymal stem cell therapy for preterm brain inflammation	Late PhD
11:25am	A29	Kyra Chan	Early administration of umbilical cord blood cells increases systemic inflammation and blood-brain barrier breakdown in injuriously ventilated preterm lambs	Early PhD
11:40am	A30	Tayla Penny	Sex differences in long-term behaviour following perinatal brain injury	Early PhD
11:55am	A31	Madeleine Smith	Do umbilical cord blood cells reduce ventilation-induced lung injury in preterm infants?	Honours
12:10pm	A32	Elliot Teo	Human placental-derived stem cells for hypoxic-ischaemic encephalopathy	Early PhD
12:20pm – 12:35pm General discussion				

12:35pm to 01:35pm Delegate Lunch (Bazar Restaurant)

Session 7 (Queenstown Room)				
Chairs: Prof Alistair Gunn and Dr Stephanie Millar				
Time	**No.**	**Speaker**		**Student/ECR**
01:35pm	A33	Kelly Zhou	The effect of ischemia and hypothermia on axonal and myelin integrity	Early PhD
01:50pm	A34	Antoniya Georgieva	Computerized analysis of intrapartum electronic fetal monitoring and hypoxic-ischemic encephalopathy at Oxford during 2012-2016	Early career
02:05pm	A35	Nathanael Yates	Ex Vivo MRI Examination of Preterm Lamb Cortical Development Following Postnatal Dexamethasone	Early career
02:20pm	A36	Kate Goasdoue	A time-course of blood-brain barrier disruption in a large animal model of neonatal hypoxic-ischemic encephalopathy	Late PhD
02:35pm	A37	Joanne Davidson	The effect of the rate of rewarming after cerebral hypothermia on post-ischemic white matter injury in the near-term fetal sheep	Not Applicable
02:50pm			General discussion	
03:05pm – 3:35pm Afternoon Tea (Foyer)				
03:35pm – 04:00pm Prize giving and Farewell				

Information for Delegates

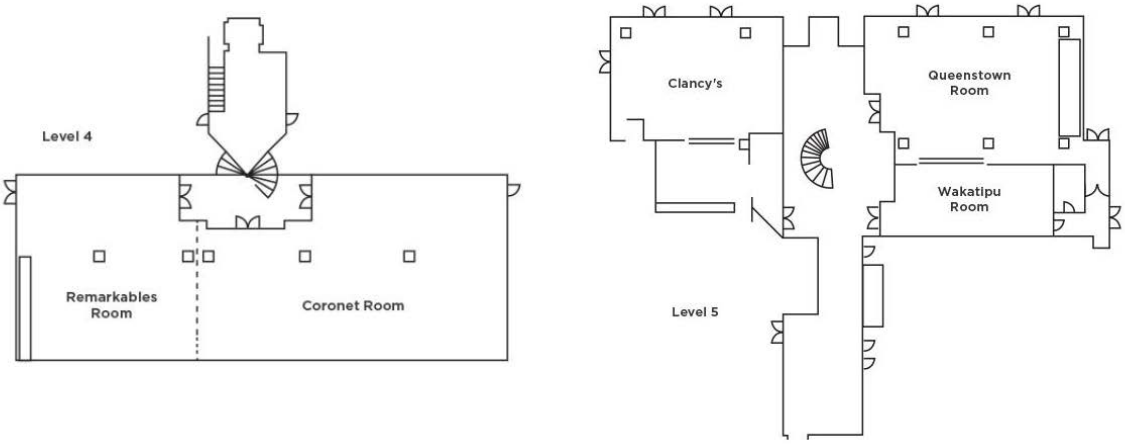
Conference location:

Rydges Lakeland Resort Queenstown

38-54 Lake Esplanade, Queenstown 9300

Rydges Conference rooms:

RYDGES
LAKELAND RESORT • QUEENSTOWN



Wifi details:

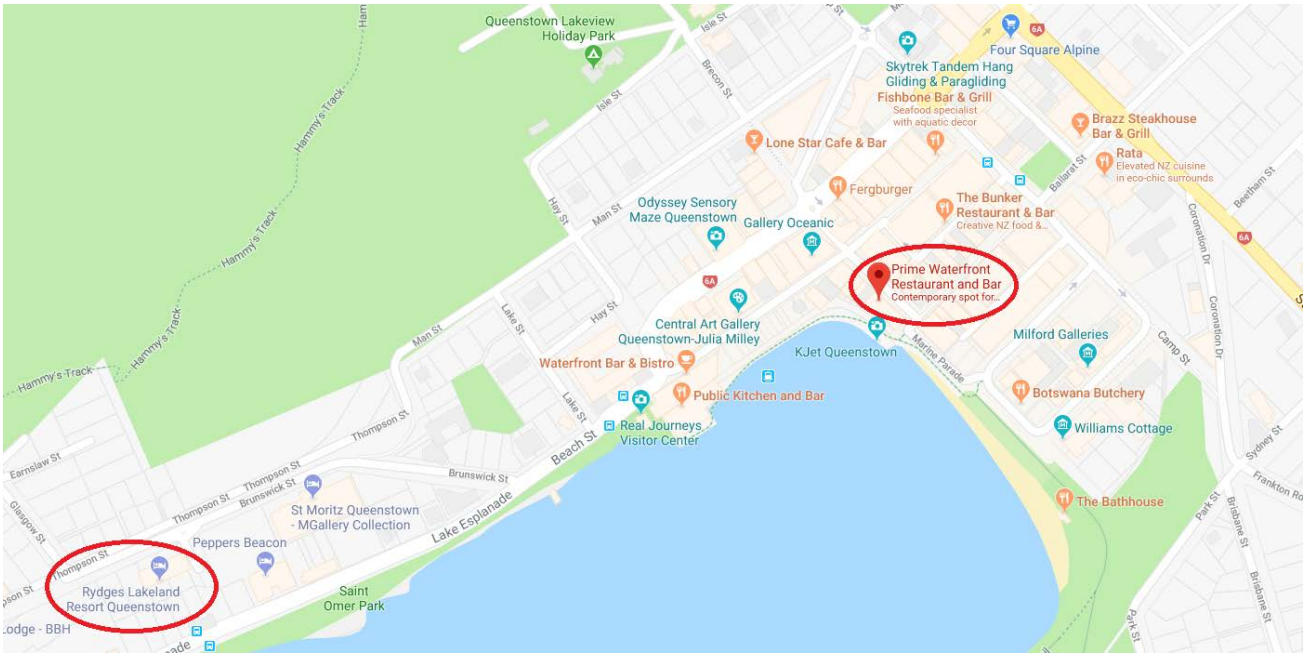
Wifi: Rydges Event

Password: bigtrout

Conference dinner location:

Prime Waterfront Restaurant and Bar

8 Rees St, Queenstown 9348



Abstracts - Session 1

Session 1 (Queenstown Room)				
Chairs: A/P Michael Stark and Prof Janna Morrison				
Time	**No.**	**Speaker**		**Student/ECR**
9:00am	A01	Deanne H. Hryciw	Role of linoleic acid in placental inflammation and cell viability	Not Applicable
9:15am	A02	Vicki Clifton	Placental glucocorticoid-regulated pathways in a sheep model of maternal allergic asthma	Not Applicable
9:30am	A03	Nadia Bellofiore	Pocket primate? The spiny mouse as a model for menstrual research	Late PhD
9:45am	A04	Harleen Kaur	A potential role for ghrelin as a driver of increased growth hormone during murine pregnancy	Early PhD
10:00am	A05	Kathryn L Gatford	Developing a fluorescent method to measure placental glucose transport in mice	Not Applicable
10:15am	OPEN		General discussion	
10:30am – 11:00am Morning Tea (Foyer)				

Role of linoleic acid in placental inflammation and cell viability

Nirajan Shrestha [1], James SM Cuffe [1], Olivia Holland[1], Anthony V Perkins [1], Andrew J McAinch [2], Deanne H. Hryciw [2, 3].

[1] School of Medical Science, Menzies Health Institute Queensland, Griffith University, Southport, QLD, Australia. [2] Centre for Chronic Disease, College of Health and Biomedicine, Victoria University, Melbourne, Australia. [3] School of Environment and Science, Menzies Health Institute Queensland, Griffith University, Nathan, QLD, Australia
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Background: The omega 6 fatty acid, linoleic acid (LA), is essential for the rapid cellular growth and development of the fetus, and can only be obtained from the diet. During pregnancy, the mother must transport LA to the fetus via the placenta. Fatty acids are transported via fatty acid-transporters (FATP) and fatty-acid binding proteins (FABP), however the specific placental proteins responsible for LA transport are unknown. In the modern diet, LA is increasing in abundance, and in non-pregnant rodents, excessive consumption of LA increases inflammation in the plasma and alters cellular viability in human umbilical vein endothelial cells. Currently, there is a paucity of research exploring the effects of increasing concentrations of LA on the placenta, which is required to gain insight into the role of LA in normal pregnancy.

Aims/Hypothesis: We hypothesise that LA will be transported by specific FATP/FABP and that elevated LA concentrations will decrease cellular viability and increase inflammatory proteins in the placenta.

Methods: The human placental trophoblast cell line (Swan 71) were treated with various physiologically relevant concentrations of LA (100, 200, 400, 500 or 1000 μM) for 24 hours. Cell viability was assessed by the 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) assay. IL-6, prostaglandins and leukotrienes were measured in the supernatant using ELISA. Gene expressions of inflammatory markers and fatty acid transport proteins were evaluated by quantitative Real-time PCR.

Results: Cell viability was significantly decreased after treatment with LA at all concentrations for 24 hours compared to vehicle control. LA significantly decreased IL-6 mRNA expression and IL-6 secretion at doses greater than 200 μM in a dose dependant manner. IL-8 mRNA expression was increased by LA at 400 μM only. A higher concentration of LA (500 μM) significantly upregulated FATP4 and FABP5, whilst FABP3 was significantly decreased by the treatment of LA at all concentrations.

Conclusions: This study demonstrated that high (supra-physiological) LA decreases placental cell viability, and alters inflammatory responses and fatty acid transporters. If LA induced similar effects in vivo, this may lead to altered placental, and therefore fetal growth and development.

Placental glucocorticoid-regulated pathways in a sheep model of maternal allergic asthma

Vicki L Clifton, Megan McDonald, Janna L Morrison, Stacy L Holman, Zarqa Saif, Ashley Meakin, Amy Woolridge, Kathryn Gatford, Megan J Wallace, Beverly S Muhlhausler, Rob Bischof, Tim Moss

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Background: Maternal asthma increases the risk of adverse pregnancy outcomes, including fetal growth restriction and neonatal diseases resulting from impaired development and maturation. In humans, maternal asthma may adversely affect fetal growth and development by altering placental expression of glucocorticoid receptor (GR) isoforms

Aim: Our aim was to examine the effect of maternal asthma on placental GR profiles and downstream signalling pathways using sheep.

Methods: Allergic asthma was induced in 7 singleton-bearing ewes before timed mating; controls were 5 singleton-bearing unsensitised (saline-treated) ewes. At 140±1 days' gestation (term is ~145d) placentomes (morphologically classified as types A-D) were collected. Placental protein and mRNA levels of GR isoforms and components of downstream signalling pathways were measured (by Western blot and qRT-PCR) in each placentome type. Data were compared between groups using SPSS.

Results: The proportion of type B, C and D placentomes in asthmatic ewes was higher than in controls. Nine known GR isoforms were detected in cytoplasmic and nuclear fractions of all placentome types. Immunoreactive proteins with molecular weights 68/69, 60, 48 and 45 kDa were detected. Placental expression of different GR isoforms was higher ($p<0.05$) in asthmatic ewes than controls in a placental type-specific manner: higher GRα-C1-3, GRα-D1 and GR A in Type A placentomes; higher GRγ, GRα-A and GR-P in type B placentomes; and higher GRα-A in type D placentomes. Placental expression of some known GR-regulated genes was higher ($p<0.05$) than control in asthmatic ewes: Type A had increased *TNFα* mRNA levels, Type B had increased *IL-1β, IFNγ, IGF2* and *LCB*3 and Type D had increased *VEGF receptors* mRNA.

Conclusions: This is the first study to show expression of different GR isoforms in the sheep placenta. Maternal asthma influences placental development in sheep, and different placentome types have different responses to maternal asthma.

Pocket primate? The spiny mouse as a model for menstrual research

Nadia Bellofiore[1,2], Shreya Rana[1,2], Fiona Cousins[1,2], Peter Temple-Smith[2] and Jemma Evans[3]

[1]The Ritchie Centre, Hudson Institute of Medical Research, Clayton, Australia; [2]Obstetrics and Gynaecology, Monash University, Clayton, Australia; [3]Centre for Reproductive Health, Hudson Institute of Medical Research, Clayton, Australia
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Background: Our discovery of the first naturally menstruating rodent, the spiny mouse (*Acomys cahirinus*) has the potential to reduce the heavy reliance on primates for menstrual related research. Validation of this species as a model requires comprehensive analysis of their fundamental reproductive biology.

Aims/Hypothesis: Our research aims to validate the use of the spiny mouse (SpM) as a rodent model of menstruation by *1)* morphological comparison during menses to the human and artificially induced mouse model of menstruation (MMoM) and *2)* behavioural analysis to assess peri-menstrual changes in SpM.

Methods: 1) Endometrial tissue from women, MMoM and the SpM during early, mid and late menstruation was collected. H&E was used to assess tissue morphology, and immunohistochemical analysis for immune cells and epithelial changes. Immunofluorescence was used to examine vascular remodelling in SpM. *2*) Virgin SpM (n=11) were subjected to Open Field (OF) and Elevated Plus Maze (EPM) tests to assess exploration and anxiety at each stage of the menstrual cycle. The late luteal and early menstrual phases were designated premenstrual phases.

Results: *1)* Induced decidualisation in MMoM is uncontrolled, but occurs spontaneously and to a lesser extent in humans and SpM. SpM menstruation resembles human "piecemeal" menstrual shedding, with focal epithelial breakdown and lysis of underlying stroma observed adjacent to regions of unshed stroma. Influx of inflammatory cells is similar across all species. *2)* Females in their early menstrual phase travelled significantly less distance in the outer zone of the OF arena (13.3 ± 9.0 m) than females in their early luteal phase (22.3 ± 9.9m) and at significantly reduced velocities (40.2 ± 10.5mm/s and 78.8 ± 31.0mm/s, respectively). Females also travelled less distance in the EPM open arms (3.2 ± 2.8m and 7.0 ± 5.5m, respectively).

Conclusions: We demonstrate for the first time that the spiny mouse shares both physiological and behavioural attributes of menstruation with the human, and may therefore be highly useful in large-scale investigation of menstruation and menstrual disorders.

A potential role for ghrelin as a driver of increased growth hormone during murine pregnancy

Harleen Kaur[1,2], Hui Li[4,5], Pamela Su-Lin Sim[3], Rebecca L Wilson[1,2], Lili Huang[6], Chen Chen[6], Johannes D Velhuis[7], Amanda Page[4,5], Beverly S Muhlhausler[3], Claire T Roberts[1,2], Kathryn L Gatford[1,2]

[1]Robinson Research Institute, The University of Adelaide, Adelaide, Australia; [2]Adelaide Medical School, The University of Adelaide, Adelaide, Australia; [3]FOODplus Research Centre, School of Agriculture, Food and Wine, The University of Adelaide, Adelaide, Australia; [4]Vagal Afferent Research Group, Centre for Nutrition and Gastrointestinal Diseases, The University of Adelaide, Adelaide, Australia; [5]South Australian Health and Medical Research Institute (SAHMRI), Adelaide, Australia; [6]School of Biomedical Sciences, University of Queensland, St Lucia, Brisbane, Australia; [7]Endocrine Research Unit, Mayo School of Graduate Medical Education, Centre for Translational Science Activities, Mayo Clinic, Rochester, Minnesota, USA.
harleen.kaur@adelaide.edu.au

Background: Circulating growth hormone (GH) levels increase during pregnancy in mice as in humans and this is important for maternal metabolic adaptations to pregnancy. Unlike humans, GH secretion during pregnancy in rodents remains pituitary-derived and the mechanisms underlying increased GH are unknown. Ghrelin, a GH secretagogue expressed in stomach, as well as the placenta, may be involved in this process.

Aims/Hypothesis: To assess changes in circulating GH profiles and ghrelin levels during murine pregnancy.

Methods: Circulating GH concentration profiles (6 h x 10 min intervals) were measured in non-pregnant female (*n*= 17), and pregnant C57BL/6J mice at 17.5 days after mating (*n*=11). Kinetics and secretory patterns of GH secretion were determined by deconvolution. Blood and tissues were collected to measure total and active ghrelin concentrations in plasma and numbers and localisation of ghrelin-positive cells in stomach and placenta.

Results: Total ($P=0.003$) and basal ($P<0.001$) GH secretion rates were higher, and orderliness of GH pulses was lower ($P=0.006$), in pregnant compared to non-pregnant mice. GH pulse frequency and mass and pulsatile secretion rate were similar in both groups. Total, but not active plasma ghrelin concentrations were higher in pregnancy ($P=0.03$). The density of gastric ghrelin-positive cells was higher in pregnant mice compared to non-pregnant mice ($P<0.001$). In addition, ghrelin protein was expressed in placental trophoblast.

Conclusions: Increased expression of ghrelin in stomach and the additional source of placental ghrelin may contribute to increased circulating total ghrelin in pregnancy. Whether this explains increased GH secretion in pregnancy is less clear, since no changes in plasma active ghrelin concentrations were observed.

Developing a fluorescent method to measure placental glucose transport in mice

Rebecca Wilson[1,2], Harleen Kaur[1,2], Beverly S Muhlhausler[3], Claire T Roberts[1,2], Kathryn L Gatford[1,2]

[1]Robinson Research Institute; [2]Adelaide Medical School; and [3]FOODplus Research Centre, School of Agriculture, Food and Wine; The University of Adelaide, Adelaide, Australia.
Kathy.gatford@adelaide.edu.au

Background: Placental nutrient transport assessment is essential in experimental models to provide a functional measure of fetal nutrient supply and uptake and their responses to interventions. In small species including mouse and guinea pig, radiolabelled tracers have been used to measure passive and active transport of solutes across the placenta. Use of fluorescent rather than radioactive labels for solutes has benefits for safety and waste disposal and potentially allows collection of additional data including time course and localisation.

Aims/Hypothesis: Measure placental glucose transport using fluorescently-labelled 2-deoxyglucose (2-DG).

Methods: Fluorescently-labelled 2-DG (IRDye800CW 2-DG, LI-COR Biosciences, Lincoln, NE) was injected into the tail vein of pregnant C57Bl6/J mice 17.5 days after mating. In initial dose and time course studies (n=5), mice were anaesthetised by intra-peritoneal injection of Avertin immediately after dye injection and the uterus exposed to allow serial live imaging under isofluorane anaesthesia (wavelengths: Ex 760 nm/Ex 790 nm, IVIS Lumina XRMS system, Perkin Elmer). In subsequent studies, mice were injected with dye then returned to the home cage for 3, 30, 60 or 120 min before anaesthesia, humane killing and tissue collection (n=2-3 for each time). Fluorescent signals were measured in whole (IVIS) and homogenised fetal and placental tissues and in placental cryosections (800 nm channel, Odyssey Imaging System, LI-COR).

Results: In intact animals under anaesthesia, uterine signal over conceptuses stabilised within 30 min of dye administration and remained stable during subsequent imaging (up to 60 min). When dams remained non-anaesthetised post dye injection, fetal signals were variable within litters collected at 3 min and consistently higher in fetuses collected at 30, 60 or 120 min, with the reverse pattern for placental signals. We are now using zone-specific auto-fluorescence of placenta at 700 nm to quantify 2-DG within junctional and labyrinth zones.

Conclusions: For the first time, dose and time course have been optimised for measuring placental glucose uptake using fluorescently-labelled 2-DG. Further validation against fetal blood flow and in IUGR is planned.

Abstracts – Session 2

Session 2 (Queenstown Room)				
Chairs: Prof Vicki Clifton and Dr Amy Woolridge				
Time	**No.**	**Speaker**		**Student/ECR**
11:00am	A06	Stephanie Miller	Identification and expression of a unique neonatal variant of the GABAA receptor alpha-3 subunit	Early Career
11:15am	A07	Jago van Dam	Potential mechanisms underlying altered LTD-like neuroplastic responses to brain stimulation in children exposed to gestational diabetes in utero	Early PhD
11:30am	A08	Sonja Brennan	The renal parenchyma - Evaluation of a novel ultrasound measurement to assess fetal renal development	Early PhD
11:45am	A09	Janna Morrison	Fetal Cardiac Haemodynamics: Initial Experience using 4D flow MRI	Not applicable
12:00pm	A10	Jeremy Lum	Maternal Methadone Treatment Causes Cognitive Deficits in Adolescent Offspring: A Rodent Study	Late PhD
12:15pm	OPEN		General discussion	
12:30pm – 1:30pm Delegate Lunch (Bazar Restaurant)				

Identification and expression of a unique neonatal variant of the GABAA receptor α3 subunit.

Stephanie Miller, Samuel Pelly, Aven Lee, Viskasari Kalanjati, Paul Colditz, Tracey Björkman.

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

Background: The $GABA_A$ receptor provides the majority of inhibitory neurotransmission in the adult central nervous system but in immature brain is responsible for much of the excitatory drive, a requirement for normal brain development. It is well known that $GABA_A$ receptor subunit expression changes during brain development.

Aims/Hypothesis: In the present study, we have identified a splice variant of the $GABA_A$ receptor α_3 subunit which appears unique to the developing brain, referred to here as the $GABA_A$ receptor α_3 subunit neonatal variant ($GABA_A$ receptor α_{3N}).

Methods: Total RNA was extracted from 91 days GA, 100 days GA (full-term = 115 days gestational age), postnatal day 4 and adult pig cortex. Primers were designed against the full coding region human of the $GABA_A$ receptor α_3 subunit. PCR products, resolved on agarose gels, were visualised and excised for further sequence analysis. Total protein samples and subcellular fractions from cortex, hippocampus, and thalamus were prepared, and protein expression analysed with Western blot.

Results: RT-PCR and sequence analysis revealed splicing of exon 8 of the α_3 subunit. Western blot analysis showed expression of $GABA_A$ receptor α_{3N} in the cortex of several neonatal species and significantly reduced expression of this splice variant in the corresponding adult brains. Expression was evident in multiple brain regions and decreased across development in the pig. Fractionation revealed differential cellular localisation in the parietal cortex, hippocampus and thalamus of the full-length $GABA_A$ receptor α_3 and $GABA_A$ receptor α_{3N}. Immunoprecipitation showed direct interaction of α_3 with the $GABA_A$ receptor subunits α_1 and γ_2 but not with gephyrin.

Conclusions: The unique presence of this variant in neonatal brain suggests an important role in development and functioning of the immature excitatory $GABA_A$ receptor system and its trophic role in development. Studies to further characterise this splice variant and its function are warranted.

Potential mechanisms underlying altered LTD-like neuroplastic responses to brain stimulation in children exposed to gestational diabetes *in utero*

Jago Van Dam[1], Mitchell Goldsworthy[1], Julia Pitcher[1]

[1]Neuromotor Plasticity and Development (NeuroPAD) – Robinson Research Institute, Adelaide Medical School, University of Adelaide, South Australia.
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Background: Children exposed to gestational diabetes mellitus (GDM) *in utero* are at increased risk of neurodevelopmental difficulties. We have provided the first neurophysiological evidence, and show that when compared with their peers, GDM-exposed children exhibit smaller and more variable LTD-like neuroplastic responses to brain stimulation, in addition to lower cortical excitability and salivary cortisol. However, recent evidence indicates the potential for plasticity-independent factors to influence the 'neuroplastic' response to stimulation, including cortisol and the relative excitability of distinct cortical circuits. Here we describe and present preliminary evidence for ongoing research exploring these mechanisms to better understand the effects of GDM on cortical and neuroendocrine development and function in adolescence.

Aims/Hypothesis: To explore potential associations between TMS-induced neuroplasticity and: the cortisol awakening response, the relative excitability of complex, oligosynaptic cortical circuits, and motor response variability. The within-subject variability of the above neurophysiological markers will also be assessed.

Methods: Transcranial magnetic stimulation (TMS) is used to evoke and measure neurophysiological responses, including neuroplasticity. Response onset latencies using various cortical current directions are used as a marker of relative excitability of distinct cortical populations (Hamada *et al.,* 2013). Cortisol is measured at 0, 15, 30, 45 minutes after awakening, and at lunch and dinner, on two consecutive days.

Results: GDM-exposed children have lower cortical excitability (η_p^2=0.199, p=0.003) and LTD-like neuroplasticity (η_p^2=0.170, p=0.005) compared with controls, which are associated with maternal diabetes severity. To date (n=19) there are no associations with response latencies. Neuroplastic responses show high intra-subject variability. Cortisol measurements are ongoing.

Conclusions: Though still unclear, we have not found evidence for an influence of variable excitability of cortical subgroups on plasticity, as seen in healthy adults. Plasticity may be the main contributor to responses, however, its variability may indicate another factor is involved – possibly altered cortisol release.

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

Sonja Brennan[1,2], Yoga Kandasamy[2,3,4], David Watson[2,5], Donna Rudd[2], Michal Schneider[5]

[1]Ultrasound Dept, Townsville Hospital, Townsville, Australia; [2]College of Public Health, Medical & Veterinary Sciences, James Cook University (JCU), Townsville, Australia; [3]Dept of Neonatology, Townsville Hospital, Townsville, Australia; [4]Mothers and Babies Research Centre, Hunter Medical Research Institute, John Hunter Hospital, The University of Newcastle, Newcastle, Australia; [5]Obstetrics & Gynaecology, Townsville Hospital, Townsville, Australia; [6]Medical Imaging & Radiation Sciences, Monash University, Melbourne, Australia.
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Background: Abnormal fetal growth can adversely impact 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 development of the fetal kidneys and provide an indirect estimate of nephron number.

Aims/Hypothesis: This study will use antenatal ultrasound to assess fetal renal parenchymal growth and determine if abnormal fetal growth affects renal parenchymal thickness. The relationship between renal parenchymal thickness, renal artery Doppler, other fetal Dopplers and amniotic fluid levels will also be evaluated. Hypotheses are – a) Renal parenchymal thickness is altered in intrauterine growth restricted (IUGR) and large for gestational age (LGA) fetuses compared to appropriately grown fetuses, b) Renal 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 and echogenicity, renal volume, fetal growth biometries, amniotic fluid measurements, renal artery Doppler and other fetal Dopplers.

Results: This study is ongoing. Currently over 75 participants have been recruited and 35 neonates delivered. Preliminary data of fetal renal parenchymal thickness and renal blood flow to birth weight will be presented.

Conclusions: Study still ongoing.

Fetal Cardiac Haemodynamics: Initial Experience using 4D flow MRI

Eric Schrauben[1], Brahmdeep Saini[1], Jack Darby[2], Jia Yin Soo[2], Mitchell Lock[2], Elaine Stirrat[1], Greg Stortz[1], John Sled[1,3], Mike Seed[1,3], Janna Morrison[2*], Christopher Macgowan[1,3*]

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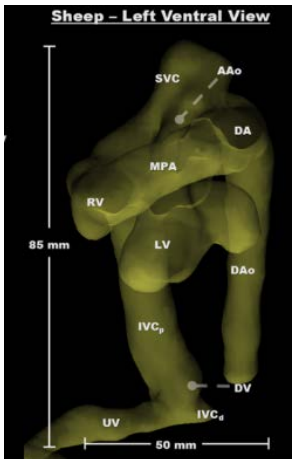

Figure 1. Example whole-heart segmentation.

Background: Visualization and quantification of complex 3D circulatory patterns within the fetal heart is difficult using conventional 2D MRI exams.

Aims: Here we apply a volumetric "4D flow" MRI [1] approach to understand cardiac haemodynamics *in utero.*

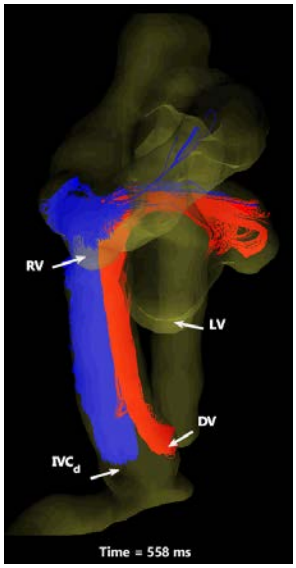

Figure 2. Fetal ventral view of oxygenated and deoxygenated blood (red and blue).

Methods: This study included 5 pregnant Merino sheep (n=5 singleton pregnancies; 130 d; term=150 days). Catheters were placed in a fetal artery to measure heart rate and trigger the MRI as previously described [2, 3]. Data was collected using a 3T MR scanner (Skyra, Siemens) and processed using research software (Siemens 4D Flow v2.4) [4]. Whole-heart assessment included segmentation, flow measurements, and visualization of fetal shunts with particle traces.

Results: Whole-heart segmentation was achieved (Fig. 1). Particle traces (Fig. 2) show right-to-left shunting of oxygenated blood. Flow values were measured, indexed to fetal weight, and used to calculate ventricular output (Table 1).

Conclusion: Here we present the first use of 4D flow MRI for comprehensive evaluation of fetal cardiac haemodynamics in a large animal model, and present evidence of unmixed streams of oxygenated and deoxygenated blood through the fetal heart.

		CVO	MPA	AAo	SVC	DA	PBF	DAo	UV	FO	IVC_d	IVC_p	DV
Mean Flow (mL/min/kg)	Sheep	383	227	144	174	183	26	245	100	85	87	214	76
	# measurements		2	2	2	2	2	4	5	3	4	5	4
Mean Flow (% of CVO)	Sheep		MPA	AAo	SVC	DA	PBF	DAo	UV	FO	IVC_d	IVC_p	DV
			59	38	45	48	7	64	26	22	23	56	20

Acronyms: UV: umbilical vein; DV: ductus venosus; IVC_d: distal inferior vena cava; IVC_p: proximal IVC; SVC: superior vena cava; RV: right ventricle; LV: left ventricle; MPA: main pulmonary artery; DA: ductus arteriosus; AAo: ascending aorta; DAo: descending aorta.

References: [1] Markl, JMRI, 2012. [2] Duan, *AJP*, 2017 [3] Duan, *JCMR* 2017. [4] Gulsun, *ISMRM*, 2012.

Maternal Methadone Treatment Causes Cognitive Deficits in Adolescent Offspring: A Rodent Study

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Background: The rate of maternal opioid use is sharply increasing, causing a greater incidence of *in utero* opioid exposure. Longitudinal studies have reported *in utero* exposure to opioids, such as methadone can cause long-term educational and neurodevelopmental deficits, well into adolescence. Furthermore, there is no adequate treatment approach for affected individuals, likely due to a limited understanding of the altered neurodevelopmental effects that underlie these behavioural deficits and lack of clinically relevant animal models.

Aims/Hypothesis: We aimed to develop a clinically relevant rodent model to investigate the effects of *in utero* methadone exposure on adolescent behaviour. We hypothesise offspring exposed to maternal methadone would display behavioural deficits, in particular cognitive dysfunction.

Methods: Female dams were treated with methadone (30mg/kg/day) via drinking water (0.2% saccharin) for a minimum of 2 week prior to mating, throughout gestation and first 2 weeks of the offspring's postnatal life. Once offspring reached adolescence (PN35), locomotor activity and recognition memory was assessed using the open field (OFT) and novel object recognition (NOR) tests.

Results: Adolescent offspring exposed to maternal methadone treatment displayed significantly reduced locomotor activity in the OFT, compared to non-exposed offspring. Furthermore, maternal methadone exposure caused significant deficits in recognition memory in the NOR test, in both male and female offspring.

Conclusions: Our findings show that perinatal methadone exposure is associated with deficits in cognitive function at adolescence, consistent with findings reported from longitudinal studies in clinical populations. Therefore, the present work may provide a clinically relevant rodent model to investigate the neurodevelopmental abnormalities caused by maternal methadone use, in order to develop advantageous treatment strategies.

Abstracts – Session 3

Session 3 (Queenstown Room)				
Chairs: Dr Kathy Gatford and Dr Guido Wassink				
Time	**No.**	**Speaker**		**Student/ECR**
01:30pm	A11	Amy Wooldridge	Relationship between size at birth and postnatal allergy	Early career
01:45pm	A12	Jack Darby	Maternal undernutrition increases IGF2 signalling molecules and fibrosis in the heart of the late gestation sheep fetus	Late PhD
02:00pm	A13	Ishmael Inocencio	Sildenafil and Dopamine for Cardiovascular support in FGR lambs	Late PhD
02:15pm	A14	Kirat Chand	Neuropathology in the IUGR piglet model	Early career
02:30pm	A15	Julie Wixey	Ibuprofen treatment to reduce inflammation and neuronal injury in the growth restricted neonatal brain	Early Career
02:45pm	OPEN	General discussion		
3:00pm – 3:30pm Afternoon Tea (Foyer)				

Relationship between size at birth and postnatal allergy

Amy Wooldridge[1,2], Manpreet Kaur[2,3], Mark McMillan[2,4], Helen Marshall[2,4], Kathryn Gatford[2]

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Background: Data from animal models and some human literature suggests that *in utero* growth restriction is protective against allergy. Variability in human literature, including in the different allergic diseases and ages assessed, has made drawing overall conclusions challenging. No existing reviews on this topic have used systematic review methodology, except for one meta-analysis of the relationship between absolute birth weight and eczema, not corrected for gestational age and limited by inclusion of studies with poorly defined outcomes.

Aim: To assess existing studies and provide clarity on the relationship between size at birth or fetal growth rate, relative to gestational age, and postnatal allergic disease (eczema, hay fever, allergic asthma, food allergy).

Methods: The protocol is published (1) and the review registered on the PROSPERO database of prospectively registered systematic reviews. We comprehensively searched 14 databases. The exposures of interest are size at birth or fetal growth, and the outcomes of interest are physician diagnosis (direct or parental-reported) or defined clinical symptoms allowing specific diagnosis of eczema, hay fever, allergic asthma and food allergy.

Results: This systematic review is still in progress. Literature searches identified 12 595 unique records published to the end of 2017. We have currently screened 73% of abstracts and titles and are currently going through full-text appraisal with 84% of full-texts screened to date (60 meet criteria for inclusion). The major reasons for exclusion at full-text screening include allergic outcomes not being analysed by size at birth or fetal growth measures (>300 records, 25%), abstract exclusion reasons (>290 records, 24%), the record is not full text (>170 records, 14%) and asthma diagnosis included non-allergic asthma (>170 records, 14%).

Conclusions: Our evaluation to date has identified important information needed to allow data synthesis and comparison including clear diagnostic criteria for allergic disease, parental allergy, and gestational age.

(1) Wooldridge *et al.* (2016) JBI Database System Rev Implement Rep 14(11):11-20

Maternal undernutrition increases IGF2 signalling molecules and fibrosis in the heart of the late gestation sheep fetus

Jack Darby[1], Caroline McMillen[1] and Janna Morrison[1]

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Background: Epidemiological studies have consistently shown that poor *in utero* conditions influence adult cardiac health. Previously, models of placental insufficiency that restrict both oxygen and glucose delivery to the fetus have identified increased hypertrophic signalling in the heart of the growth restricted fetus.

Aim: We aimed to determine the effect of decreased fetal nutrient supply in late gestation normoxemic fetuses.

Methods: At 115 days (d) gestation (term=150d), ewes were randomly divided into either a control or an undernutrition (LGUN) group that received a 50% reduction in nutrient intake until 145d gestation. Fetal blood samples were collected across late gestation for blood gas and glucose analysis. Right ventricle tissue was collected at post mortem (145d).

Results: There was evidence of an up-regulation of IGF2/IGF2R signalling through the CAMKII pathway in the fetal right ventricle in the LGUN group. LGUN increased expression of *COL1A (Fig1)*, *TIMP1* and *TIMP3* in the right ventricle of the fetal heart. The presence of interstitial fibrosis in the heart of the LGUN group was confirmed through the quantification of picrosirius red stained sections of the right ventricle.

Conclusions: We have shown that maternal undernutrition in late gestation may drive the onset of myocardial remodelling in the fetal right ventricle and thus have negative implications for right ventricular function and cardiac health in later life.

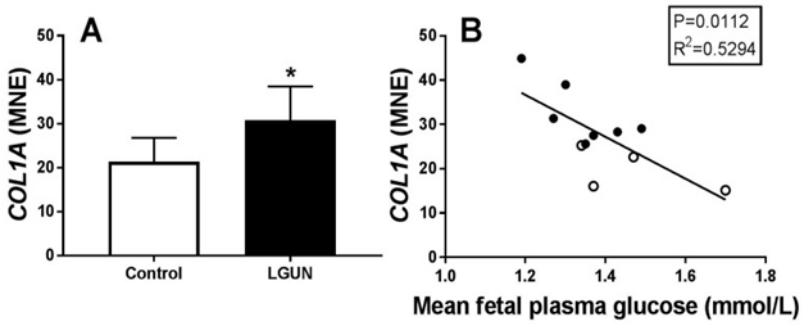

Fig1*. LGUN increased the mRNA expression of COL1A (A) in the fetal right ventricle and this was positively correlated with mean fetal plasma glucose concentrations*

Sildenafil and Dopamine for Cardiovascular support in FGR lambs

Ishmael Inocencio [1], Graeme Polglase[1], Ilias Nitsos[1], Valerie Zahra[1], Suzanne Miller[1], Beth Allison[1].

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Background: The transition from fetal to neonatal life requires immediate structural and functional cardiovascular (CV) adaptations. Growth restricted (FGR) infants mount *in-utero* CV adaptations to survive adverse uterine conditions, which result in post-natal consequence that contribute to greater CV instability and mortality in early and later life. Preterm FGR infants require greater CV support in the first week of life compared to appropriately grown controls (Sengal 2016). We have shown that the nitric-oxide NO pathway is dysfunctional both before and after birth, following FGR (Polglase 2016). NO dysfunction in FGR fetuses may underlie the CV instability in the first days and weeks of life. Sildenafil modulates the NO pathway by increasing NO bioavailability. We hypothesise that postnatal administration of sildenafil will improve NO bioavailability and provide greater CV support than the current clinical treatment dopamine

Aims/Hypothesis: To compare the efficacy of sildenafil vs. dopamine to improve CV function in FGR preterm lambs.

Methods: Preterm lambs (0.6 gestation) underwent sterile surgery for single umbilical artery ligation (SUAL, FGR) to induce FGR or sham (control, AG) surgery. Fetus underwent caesarean section at 0.8 gestation and were instrumented to measure pulmonary, cardiovascular and cerebral pressures and flows. Lambs were then delivered and ventilated for 4 hours. One hour post-delivery lambs were randomised to receive i.v sildenafil citrate (SC (dose), dopamine (DOP dose) or vehicle. Physiological and ventilator parameters and oxygenation were continuously monitored throughout the study.

Results: FGR lambs were 18% smaller than AG lambs (p=0.005). Dopamine treatment decreased heart rate and blood pressure in FGR_{DOP} compared to AG at 4hr (p=0.0435 and p= 0.0183 respectively). Treatment with SC did not result in different HR and BP compared to AG control.

Conclusions: While sildenafil administration did not alter cardiovascular function in FGR, CV detriment was observed after dopamine treatment. This data suggest dopamine may not be an appropriate treatment for FGR infants.

Neuropathology in the IUGR piglet model

Kirat Chand, Stephanie Miller, Kate Goasdoue, Paul Colditz, Stella Tracey Bjorkman, Julie Wixey

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Background: Intrauterine growth restriction (IUGR) is the second leading cause of perinatal morbidity and mortality. IUGR is commonly caused by placental insufficiency, resulting in an inadequate supply of oxygen and nutrients to the fetus. The fetal brain is particularly vulnerable to IUGR conditions evidenced by neuronal and white matter injury and abnormal neurodevelopment in the IUGR infant. Our group utilises the piglet as a model of IUGR as growth restriction occurs spontaneously in the pig, making it a highly relevant model of human IUGR. The piglet brain closely resembles the developmental stage of a human newborn, in terms of size, structure and functional development with a similar grey to white matter ratio to that of the human infant. The neuropathology in our piglet model has not yet been thoroughly investigated.

Aims/Hypothesis: Here we characterised the neuropathology of the IUGR neonatal brain in our preclinical piglet model.

Methods: Newborn IUGR (<5th centile) and normally grown piglets were euthanised on postnatal day 0 (P0 – day of birth) or P4. We investigated neuropathology using cellular markers such as Iba-1 (microglia), GFAP (astrocytes), NeuN (neurons) and Luxol Fast Blue (white matter/myelination status).

Results: We observed significant increases in the number of both microglia and astrocytes in the parietal cortex and white matter in IUGR piglet brain on P0 and P4 compared with controls. These increases were associated with a change in activation state, as noted by altered glial morphology. The IUGR brain also displayed lower neuron counts and myelination index, indicating neuronal loss and white matter injury.

Conclusions: Our findings suggest that our piglet model of IUGR displays the characteristic neuropathological outcomes similar to those reported in the IUGR human and other IUGR animal models. The activated glial morphology indicates altered inflammatory responses which may be related to neuronal loss and white matter disruption. These findings support the use of our model in studying brain outcomes in IUGR neonates.

Ibuprofen treatment to reduce inflammation and neuronal injury in the growth restricted neonatal brain

Julie Wixey, Kishen Sukumar, Stephanie Miller, Kate Goasdoue, Paul Colditz, Stella Tracey Bjorkman, Kirat Chand

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Background: Intrauterine growth restriction (IUGR) is a condition where the fetus does not achieve optimal growth, commonly caused by placental insufficiency. The chronic decrease in blood flow restricts oxygen and nutrient supply to the fetus damaging numerous organs with the fetal brain being particularly vulnerable. Neuronal and white matter injury are evident in IUGR infants and it has recently become evident that inflammation may be a key mechanism responsible for the progression of this injury. We will investigate whether the use of an anti-inflammatory (ibuprofen) could reduce inflammation and neuronal and white matter injury in the IUGR neonatal brain.

Aims/Hypothesis: We hypothesise ibuprofen treatment will reduce neuroinflammation and neuronal and white matter injury in the IUGR piglet brain.

Methods: Newborn IUGR (<5th centile) and normally grown control piglets were monitored, fed and cared for until euthanasia on postnatal day 4 (P4). 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. Markers of inflammation (microglia (Iba-1) and astrocytes (GFAP)), neuronal injury (NeuN) and white matter injury (Luxol Fast Blue) were examined using immunohistochemistry. Proinflammatory cytokines were examined using PCR array profilers.

Results: Ibuprofen treatment reduced the numbers of microglia and astrocytes in the parietal cortex of the IUGR piglet brain on P4 as well as decreasing proinflammatory cytokines. Ibuprofen treatment also reversed the reduction in neuronal cell counts and myelin index apparent in the parietal cortex of IUGR piglets.

Conclusions: Our findings suggest ibuprofen reduces the inflammatory response in the IUGR neonatal brain and concurrently reduces neuronal and white matter injury. Further research will determine whether ibuprofen's action is systemic or central and whether treatment has a long-lasting neuroprotective effect.

Abstracts – Session 4

Session 4 (Queenstown Room)				
Chairs: Prof Laura Bennet and Dr Christopher Lear				
Time	**No.**	**Speaker**		**Student/ECR**
03:30pm	A16	Vivian Tran	The effects of preterm birth and intrauterine inflammation on the structure of the aortic wall	Early PhD
03:45pm	A17	Bianca Le	Cardiac ventricular structure at 2 and 5 months after birth in lambs born very preterm and ventilated in the neonatal period	Late PhD
04:00pm	A18	Jia Yin Soo	The interaction between fetal growth, preterm birth and methamphetamine exposure on drug metabolism	Late PhD
04:15pm	A19	Mitchell Lock	Immediate gene response following myocardial infarction in the fetal and adolescent sheep heart	Late PhD
04:30pm	A20	Victoria King	Effect of chronic inflammation on circadian rhythms in the preterm fetus	Masters
4:45pm	A21	Simerdeep Dhillon	Cardiovascular effects of delayed human recombinant erythropoietin treatment after asphyxia in preterm fetal sheep	Late PhD
05:00pm	OPEN	General discussion		
07:00pm to 10pm Delegate Dinner (Prime Restaurant)				

The effects of preterm birth and intrauterine inflammation on the structure of the aortic wall

Vivian Tran[1], Stacey J Ellery [2], Megan R Sutherland[1], Antony Vinh[4], Amanda Vrselja[1], Grant R Drummond[4], Tim J Moss[2], J Jane Pillow[3] and M Jane Black[1]

[1]Monash University, Clayton, Australia, [2]Hudson Institute of Medical Research and Monash University, Clayton, Australia, [3]University of Western Australia, Crawley, Australia, [4]LaTrobe University, Bundoora, Australia.
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Background: Intrauterine inflammation (chorioamnionitis) is the most common cause of preterm birth. Preterm birth and chorioamnionitis may adversely impact the immature fetal/neonatal vasculature.

Aims/Hypothesis: We aimed to determine the effect of preterm birth, with or without exposure to intrauterine inflammation, on the structure of the thoracic aorta in the early neonatal period, using a clinically relevant sheep model.

Methods: Saline or lipopolysaccharide (LPS; 4mg, to induce intrauterine inflammation) was administered to pregnant ewes 48 h prior to preterm delivery via intra-amniotic injection. Preterm + saline (n=9). Maternal intramuscular betamethasone (5.7 mg) was given 6 h and 24 h post LPS. Preterm + LPS (n=10) lambs were delivered at 128 d gestation (term=147 d), managed according to contemporary neonatal practice and euthanised at postnatal day 7. Age-matched fetal controls were delivered and euthanised at 135 d gestation (n=7). The wall composition of the thoracic aorta was examined histologically, and oxidative stress was assessed by measuring oxidative protein modification (3-NT by IHC). The mRNA expression of markers of oxidative stress and endothelial injury were assessed using Fluidigm.

Results: Compared to fetal controls, preterm +LPS lambs had a significantly reduced aortic lumenal area (p=0.04), with a trend towards a reduction also in the preterm + saline lambs (p=0.058); there were no differences in thoracic aortic wall composition (collagen, elastin, and smooth muscle content). Preterm aortae showed evidence of oxidative stress (nitrotyrosine staining), with a significant reduction in NOX1 mRNA expression in the preterm + LPS lambs compared to the fetal controls.

Conclusions: Preterm aortae exhibited oxidative stress, but paradoxically a reduction in NOX1 expression. Preterm birth also led to a reduction in thoracic aortic lumen area; if this persists, it may predispose to the development of vascular disease.

Funding/Support: NHMRC 1057514, 1057759, 1077691, TPCHRF, Chiesi Farmaceutici S.p.A (poractant alfa), Fisher & Paykel Healthcare (ventilator circuits), ICU Medical (monitoring lines).

Cardiac ventricular structure at 2 and 5 months after birth in lambs born very preterm and ventilated in the neonatal period

Bianca Le[1], Megan Sutherland[1], Mar Janna Dahl[2], Kurt Albertine[2], Mary Jane Black[1]

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Background: Preterm birth (delivery prior to 37 weeks of gestation) occurs in 11% of births worldwide. Preterm infants are born at a time when their hearts are structurally immature. Studies in lambs born late preterm show cardiac remodelling in the neonatal heart.

Aims/Hypothesis: We hypothesised that the structural changes in the myocardium following preterm birth in lambs would be more severe when the gestational age at birth is decreased and assisted postnatal ventilation is required. The aim of this study was to determine the impact of preterm birth on the structure of the left ventricle at 2 and 5 months of age in lambs born very preterm and exposed to assisted ventilation postnatally.

Methods: Lambs were delivered preterm via caesarean section at 128 days of gestation and mechanically ventilated after birth. The lambs received antenatal dexamethasone, exogenous surfactant and caffeine citrate to facilitate lung maturation and function. Lambs were euthanised at 2 (n=8) or 5 (n=9) months term-equivalent age (TEA). Preterm lambs were compared to unventilated age-matched lambs that were born spontaneously at term (150 days gestation; n=19). Cardiomyocyte number, apoptosis, proliferation and myocardial fibrosis were analysed using immunohistochemical, histological and stereological techniques.

Results: Absolute and relative heart weight did not differ between preterm and term lambs at 2 and 5 months TEA. Interstitial fibrosis within the left ventricle was significantly greater in preterm lambs compared to term lambs ($p = 0.0006$), and greater at 5 months compared to 2 months TEA ($p = 0.0015$). Levels of cardiomyocyte proliferation and apoptosis in both ventricles were negligible in both preterm and term lambs at 2 and 5 months TEA. The number of cardiomyocyte nuclei in the left ventricle did not differ between term and preterm groups ($p=0.26$) nor between the age groups ($p=0.22$).

Conclusions: Findings from this ongoing study provide a greater understanding of preterm birth and mechanical ventilation on cardiac structure and cardiomyocyte growth.

The interaction between fetal growth, preterm birth and methamphetamine exposure on drug metabolism

Jia Yin Soo[1,2], Michael Wiese[2], Rebecca Dyson[3], Clint Gray[3], Andrew Clarkson[4], Mary Berry[3]* and Janna Morrison[1,2]*

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Background: Admission of pregnant women to hospital due to methamphetamine (MA) abuse tripled from 1994 to 2006 [1]. Studies show MA use during pregnancy is associated with preterm birth and low birth weight (LBW) [2], however poor prenatal care and nicotine are confounding factors. Drug use, LBW and preterm birth also induce hormonal changes that may affect cytochrome P450 enzyme activity and drug metabolism.

Aims/Hypothesis: To determine the separate effects of LBW, preterm birth and MA abuse during pregnancy on hepatic drug metabolism in postnatal life.

Methods: We used a combination of *in vivo* and *ex vivo* methods in guinea pigs and sheep in this study. LBW was induced using carunclectomy in sheep. Preterm birth was induced in guinea pigs at 62 d (term, 69d). Liver was collected from the LBW lambs (21d old) and preterm birth pups (28d old), microsomes extracted and analysis of dextromethorphan (CYP2D6) and Midazolam (CYP3A4). Caffeine (CYP1A2) and midazolam were orally administered to MA exposed female guinea pigs of reproductive age and pups exposed to methamphetamine *in utero* (0d old and 28d old). In all 3 studies, parent drug and metabolites were measured using Tandem Mass Spectrometry (LC-MS/MS).

Results: Using microsomes, we found that low birth weight reduced CYP3A4 enzyme activity. However, there was no effect of preterm birth on the enzyme activity of CYP2D6 or CYP3A4. Methamphetamine exposure increased CYP1A2 activity in non-pregnant female guinea pigs of reproductive age. However, *in utero* methamphetamine exposure did not affect CYP1A2 or CYP3A4 enzyme activity in 0 or 28 day old pups.

Conclusions: This study suggests that there is a differential effect of methamphetamine abuse, preterm birth and LBW on cytochrome P450 enzyme activity and this may have implications for the therapeutic profile of drugs in these offspring

Immediate gene response following myocardial infarction in the fetal and adolescent sheep heart

Mitchell Lock[1], Jack Darby[1], Jia Yin Soo[1], Ross Tellam[1], Doug Brooks[2], Enzo Porrello[3], Mike Seed[4], Joseph Selvanayagam[5], Christopher Macgowan[4], Maureen Keller-Wood[6], Charles Wood[7], Janna Morrison[1]

[1]Early Origins of Adult Health Research Group and [2]Mechanisms in Cell Biology and Disease Research Group, University of South Australia, Adelaide, Australia; [3]Cardiac Regeneration Laboratory, Murdoch Childrens Research Institute, Melbourne, Australia; [4]The Hospital for Sick Children, Toronto, Canada; [5]Flinders University, Adelaide, Australia; [6]Department of Pharmacodynamics, and [7]Department of Physiology and Functional Genomics, University of Florida, Gainesville, USA.
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Background: Animal models indicate that there are critical molecular mechanisms that can be activated to induce myocardial repair at specific points in development. Studying a large animal where the timing of heart development in relation to birth, size and electrophysiology are similar to humans may provide vital insights into the repair capacity of the developing mammalian heart and its application to the adult heart disease.

Aims/Hypothesis: We hypothesised that there would be different responses to cardiac infarction in fetal and adolescent sheep because of their differential capacities for tissue repair.

Methods: We used a sheep model of myocardial infarction induced by ligating the left anterior descending coronary artery. Surgery was performed on fetuses (at 100 days gestation when all cardiomyocytes are mononucleated and proliferative) and adolescent sheep (at 6 months of age when all cardiomyocytes contribute to heart growth by hypertrophy). Sheep were humanely killed three days post-infarction. RNA was extracted from heart tissue and gene expression was determined using an ovine specific gene array (ovine 019221 arrays; GPL14112 platform).

Results: Significantly dysregulated genes were assessed for Gene Ontology (GO) term enrichments using DAVID. Gene ontology for fetuses showed enrichment for terms such as *PI3K-Akt signaling pathway* and *TNF signalling pathway*, adolescent animals showed enrichment for terms such as *NF-kappa B signaling pathway* and *apoptosis*.

Conclusions: Fetuses demonstrated opposite regulation of a number of genes compared to adolescent sheep. A larger number of dysregulated genes were present in the adolescent sheep compared to fetal sheep indicating a 'resistance' to damage in the fetuses.

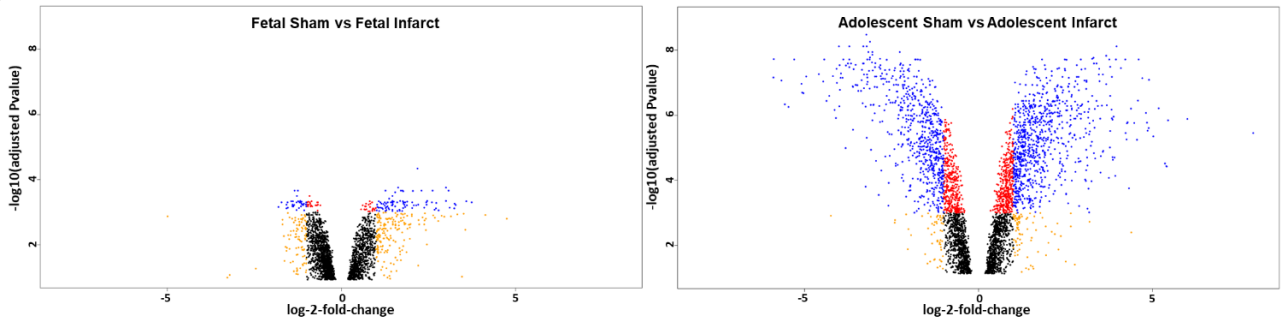

Figure 1: Volcano Plots of significantly (P<0.05) dysregulated genes in the Fetal Sham compared with Fetal Infarct and Adolescent Sham compared with Adolescent Infarct. Orange, log2 fold change >1; Red, adjusted P value<0.001; Blue, log2 fold change >1 and adjusted P value<0.001.

Effect of chronic inflammation on circadian rhythms in the preterm fetus

Victoria King, Simerdeep Dhillon, Christopher Lear, Robert Galinsky, Lotte Van den Heuij, Alistair Gunn, Laura Bennet

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Background: Circadian rhythms are important regulators of physiological activity, but little is known about fetal patterns, particularly during preterm life, and whether they are altered by infection/inflammation.

Aims/Hypothesis: To determine the effect of lipopolysaccharide (LPS) on circadian rhythms of preterm fetuses.

Methods: Preterm fetal sheep were given a 5-day saline (n=8) or LPS (n=7) infusion (ramping dose doubled/day from 200ng) at 104d and studied for a further 5 days post-infusion. Fetal heart rate (FHR), mean arterial blood pressure (MAP), and electroencephalographic (EEG), power and spectral edge frequency (SEF) were continuously measured. Light period was between 06.00-18.00hrs.

Results: In controls, MAP increased and FHR fell over the duration of the experiment. MAP increased during the day, plateaued ~18.00-02.00h, then dipped (0.2-0.8mmHg) until 06.00hrs. FHR increased from 12.00-19.00h, plateaued ~19.00-22.00h, then fell until 06.00h (~28bpm), with mixed FHR 06.00-12.00h. LPS infusion suppressed the rise in MAP, and post-infusion MAP increased day and night. LPS did not affect FHR, other than a brief tachycardia occurred at infusion onset. EEG power increased throughout and SEF increased until 108d, then plateaued and fell. EEG power increased only during the day (high voltage, low frequency), while SEF increased only at night (high frequency, low amplitude). LPS suppressed EEG power vs. controls throughout, but there was some catch up day and night activity post-infusion. LPS suppressed SEF day 1 of infusion, but had no effect on circadian activity. However, SEF did not transition at 108d, but rather continued to increase.

Conclusions: MAP and EEG power are surrogate measures of growth suggesting fetal growth occurs primarily during the day, consistent with when ewes mainly eat. Increased glucose may also support neural network development of long-distance (e.g. thalamocortical) connections that are energy intensive. Increased SEF at night may reflect a switch in energy use to local/regional neural network development. Thus, LPS affects both body and brain growth with some catch-up post infusion, and may impact on long-distance connections, but local neural network development may be sustained. This is consistent with neural network development after chorioamnionitis. Failure of SEF to transition at 108d may reflect delayed sleep state maturation.

Cardiovascular effects of delayed recombinant human erythropoietin treatment after asphyxia in preterm fetal sheep

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Background: Recombinant human erythropoietin (rEpo) has been shown to have neuroprotective potential in preclinical studies. Two large ongoing trials of rEpo in preterm infants are now testing whether 1000 IU/Kg rEpo every 48 hours is neuroprotective. However, the cardiovascular effects of repeated exposure to high dose rEpo in conjunction with perinatal insults such as asphyxia is not known.

Aims/Hypothesis: To examine the cardiovascular and cerebrovascular effects of a clinically relevant protocol of delayed administration of rEpo after asphyxia in preterm fetal sheep.

Methods: Chronically instrumented preterm (0.7 gestation) fetal sheep received sham asphyxia (n = 8) or asphyxia induced by complete umbilical cord occlusion for 25 minutes. Fetuses were then given intravenous (IV) bolus injections of saline (n = 8) or 5000 IU rEpo (n = 6) starting 6 hours after asphyxia, and then every 48 hours until 102 hours after asphyxia. Physiological recovery was monitored for seven days.

Results: IV bolus injection of 5000 IU rEpo was associated with area under the curve for plasma Epo concentration similar to that reported in preterm infants receiving 1000 IU/Kg rEpo in the ongoing clinical trials. rEpo treatment was associated with a significant increase in mean arterial pressure compared to asphyxia-saline from 24 to 48 hours ($P < 0.05$) after asphyxia. Carotid blood flow in the rEpo group was significantly lower ($P < 0.05$) than the asphyxia-saline group from 3 hours after the first rEpo bolus until the end of the recovery period. Similarly, recovery of femoral blood flow after asphyxia was also suppressed ($P < 0.05$) from 76 hours onwards. Every rEpo bolus injection was associated with transient differential changes in carotid and femoral blood flows.

Conclusions: Repeated boluses of rEpo after asphyxia altered the haemodynamic responses during recovery in preterm fetal sheep. Speculatively, prolonged reductions in carotid blood flow might be associated with an adverse effect on neural recovery after asphyxia. These preliminary data suggest the need for further preclinical examination of the effect of different doses and treatment regimens of rEpo on neural and cardiovascular outcomes after asphyxia.

Abstracts – Session 5

Session 5 (Queenstown Room)				
Chairs: Prof Rosemary Horne and Dr Rebecca Dyson				
Time	**NR**	**Speaker**		**Student/ECR**
08:00am	OPEN	Registration		
09:00am	A22	Yogavijayan Kandasamy	Evidence of glomerular injury in preterm neonates	Not applicable
09:15am	A23	Dominique Blache	Neurodevelopmental outcomes after postnatal dexamethasone in preterm lambs	Not applicable
09:30am	A24	Donna Rudd	Discovering the faecal microbiome of pre-term neonates at the Townsville Hospital NICU: First hesitant steps and preliminary findings	Not applicable
09:45am	A25	Christopher Lear	Long-term neurophysiological and histological outcomes after preterm fetal hypoxia-ischemia	Early career
10:00am	A26	Guido Wassink	Can treatment with clonidine reduce white matter damage in asphyctic preterm babies?	Early Career
10:15am	A27	Veena Kurup	Long-term effects of low dose dexamethasone and ventilation therapies on alveolar development in preterm lambs	Early PhD
10:25am	OPEN	General discussion		
10:40am – 11:10am Morning Tea (Foyer)				

Evidence of glomerular injury in preterm neonates

Yogavijayan Kandasamy [1,2,3], Donna Rudd[3] , Roger Smith[2], Eugenie R Lumbers[2,4] , Ian MR Wright[2,5]

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Background: Microalbuminuria is an early indication of glomerular pathology. Nephrin is a transmembrane protein expressed in glomerular podocytes. Nephrinuria occurs early in glomerular injury, often preceding albuminuria, and there is a positive correlation between nephrinuria and the severity of renal diseases.

Aims/Hypothesis: We carried out a study to determine the impact of prematurity on renal development. The primary outcomes measured were nephrinuria and albuminuria; renal volume and glomerular filtration rate were the secondary outcomes.

Methods: Preterm neonates born < 28 weeks of gestation, with birth weight between 10th and 90th centile (appropriate for gestational age) were recruited and underwent assessments at 28, 32 and 37 weeks postmenstrual age (PMA). During each assessment, the neonates underwent urine analysis for albumin:creatinine (ACR) and nephrin:creatinine (NCR) ratio measurements.

Results: 53 premature and 31 term neonates were recruited. The median gestational age of the premature cohort was 26.4 [24.7-27.4] weeks, with a mean birth weight of 886(179) g. There was a statistically significant decline in NCR from 32 - 37 weeks PMA (0.07[0.04-0.18] to 0.04[0.03-0.09] g/mol; P=0.028). However, there was no significant difference between NCR at 37 weeks PMA and levels in term neonates (0.04[0.03-0.09] vs 0.05 [0.03-0.14] g/mol; P=0.42). There was also a statistically significant in decline of ACR from 32-37 weeks PMA (24.5[14.5-43.5] to 6.7[4.2-23.8] g/mol; P=0.0009). ACR at 37 weeks PMA was however significantly higher compared to controls (6.7[4.2-23.8] vs 5.1 [1.9-6.8] g/mol; P=0.022).

Conclusions: The data shows evidence of glomerular injury in the early neonatal period as demonstrated by nephrinuria and albuminuria. This may be due to a combination of abnormal glomerular development, ongoing ischaemia and the use of potentially nephrotoxic antibiotics. By term equivalence, nephrinuria returns to normal but albuminuria remains abnormal.

Neurodevelopmental outcomes after postnatal dexamethasone in preterm lambs

Stephanie Clarnette, Dominique Blache, Jane Pillow

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Background: The fear of the potential adverse impact of high dose postnatal dexamethasone on neurodevelopment has limited contemporary use of dexamethasone in preterm infants to exceptional cases of prolonged ventilator dependence. Currently, a lower dose of dexamethasone is prescribed (e.g., DART protocol) that has positive respiratory effects, but the effect of low-dose dexamethasone on neurodevelopmental impairment remains unclear.

Aims/Hypothesis: To investigate the independent effects of preterm birth and low-dose dexamethasone on memory, learning fearfulness and temperament in lambs.

Methods: We conducted a double-blind, randomised controlled study that aimed to investigate further the independent neurodevelopmental outcomes of preterm birth. Surgically delivered preterm lambs (128 d gestation) were randomised to commence postnatal dexamethasone (n = 11, Dart protocol) or placebo (n = 13, Saline) at 72 h postnatal age. Term lambs (n = 6) delivered naturally at 147-150 d gestation. Cognition, temperament and behavioural activity were tested at two months post term gestation, using a battery of behavioural tests, including an open-field, novel object, mirror and maze test.

Results: Preterm lambs had reduced fearfulness, novelty seeking and behavioural activity but similar learning and memory performances compared to Term lambs ($p < 0.05$). In preterm lambs, low-dose postnatal dexamethasone had no effect on cognitive performance, fearfulness, novelty seeking or behavioural activity but reduced the pseudo social interactions during the mirror test ($p < 0.01$), compared to the placebo group.

Conclusions: Overall, our results suggest that dexamethasone administered according to the DART protocol has very limited effect on the neurodevelopment of the lambs, when assessed at two months of age.

Funding/support: NHMRC GRT1057759, GRT1057514, RF1077691; Chiesi Farmaceutici S.p.A. (poractant alfa); Fisher & Paykel Healthcare (circuits), ICU Medical (monitoring lines).

Discovering the faecal microbiome of pre-term neonates at the Townsville Hospital NICU: First hesitant steps and preliminary findings

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Background: From the perspective of a Neonatal Intensive Care Unit (NICU) it is important to understand neonatal microbiome and its assembly immediately following birth in order to tailor treatment regimens. Understanding and preserving this delicate microbial ecosystem through evidence-based interventions could be an important link to improving health outcomes for these babies.

Aims/Hypothesis: As part of a larger study, developing an understanding of the neonatal microbiome caused by pre-term birth and probiotic interventions, this pilot study was designed to develop protocols for the collection, transport and analysis of faecal samples.

Methods: All pre-term babies (<32 weeks and >32 weeks) were recruited from the THHS NICU (Oct – Dec 2017). Faecal samples were collected during the first 3 days and just prior to discharge. The microbiome was identified using both culture dependent and independent methods. DNA extraction was optimised (Bioline Isolate Faecal DNA kit) and amplification/16s library preparation was performed using two PCR cycles using 785F/800R primer combination targeting V3 and V4 regions (Illumina MiSeq System) to identify individual microbiome make up for each neonate at each time point.

Results: The sequencing of the16s DNA coding for the ribosomal 16s RNA (16S meta-barcoding) provided reproducible data. Where DNA extraction was unsuccessful, all samples showed no growth on traditional culture analysis. Intra individual variation in microbiota was seen between babies and the microbial diversity increased upon discharge. We have established an effective protocol for the collection, transport and analysis of faeces from pre-term neonates within the NICU at THHS.

Conclusions: This study confirms and validates current collection and analysis protocols. Information gained from this study will contribute to the current knowledge and clinical practices undertaken to preserve the fragile microbial ecosystem of babies admitted to NICU and therefore improve the health and quality of life for these babies.

Long-term physiological and histological outcomes after hypoxia-ischemia in preterm fetal sheep

Christopher Lear,[1] Johanna Lloyd,[1] Jialin Sae-Jiw,[1] Benjamin Lear,[1] Yoshiki Maeda,[1] Michael Beacom,[1] Joanne Davidson,[1] Alistair Gunn,[1] Laura Bennet[1]

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Background: Perinatal hypoxia-ischemia (HI) remains an important contributor to neurodevelopmental impairment after preterm birth. HI triggers acute brain injury, followed by abnormal brain development and ultimately impaired neural function. The time-course of changes is poorly understood.

Aims/Hypothesis: To examine the effects of mild or severe HI on neurophysiological and histological outcomes over 3 weeks.

Methods: At 0.7 of gestation, chronically instrumented singleton sheep fetuses received either 25 min (severe HI, n=8) or 15 min (mild HI, n=8) of complete umbilical cord occlusion, or sham occlusion (n=10). Fetal EEG and ECG activity were continuously recorded. Post-mortems were performed after 3 weeks for histology.

Results: 25 min HI was associated with reduced brain weight ($p<0.05$), loss of striatum neurons ($p<0.05$) and a limited reduction in the myelin constituent protein CNPase (2-4%) ($p<0.05$) in the intragyral and periventricular white matter regions. 15 min HI was associated with an intermediate loss of striatal neurons and CNPase labelling. HI did not affect the total number of oligodendrocytes or myelin basic protein. 25 min HI was associated with reduced EEG power over the final 7 days of recovery ($p<0.05$), but there was no effect on spectral edge frequency. Very low, low and high frequency FHRV were significantly reduced over the final 7 days of recovery after 25 min HI ($p<0.05$). In contrast, 15 min HI did not alter EEG or FHRV parameters compared to sham controls.

Conclusions: These data show good long term recovery of histological and neurophysiological outcomes after mild HI, whereas severe HI was associated with impaired EEG and FHRV maturation. Surprisingly, we only observed minimal effect on myelination in the intragyral and periventricular regions after severe HI, suggesting that impaired EEG maturation was not due to impaired myelination.

Can treatment with clonidine-hydrochloride reduce white matter damage after severe asphyxia in preterm fetal sheep?

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Background: Perinatal asphyxia is a significant contributor to death or adverse neurodevelopmental outcomes in preterm babies, for which no therapeutic treatment is available. Catapres (clonidine-hydrochloride), an alpha$_2$-adrenergic agonist with potential anti-excitotoxic properties, is used clinically to treat hypertension and attention-deficit hyperactivity disorder, and provides neuroprotection after hypoxia-ischemia in neonatal studies. However, there is limited evidence whether catapres can protect white matter in the post-asphyxial preterm brain.

Aims/Hypothesis: This study tested the effect of low- and high-dose clonidine-hydrochloride (catapres), infused from 15 minutes until 4 hours after severe asphyxia, on white matter damage in preterm fetal sheep.

Methods: Preterm fetal sheep (0.7 gestation) received sham-asphyxia (sham, n=9) or severe asphyxia induced with umbilical cord occlusion for 25 min, followed with constant infusion of saline-vehicle (occl-vehicle, n=10), or clonidine-hydrochloride at 10 mg/kg/h (sham-low-clon, n=7; occl-low-clon, n=6) or 100 mg/kg/h (sham-high-clon, n=6; occl-high-clon, n=8) from 15 minutes until 4 hours after cord occlusion. Fetuses were killed at three days.

Results: Umbilical cord occlusion or low-dose clonidine infusion was associated with substantial loss of total number of Olig2-positive oligodendrocytes in periventricular and intragyral parasagittal white matter ($P<0.05$; vs. sham) after three days recovery. Continuous infusion with low or high-dose clonidine-hydrochloride after umbilical cord occlusion did not improve Olig2-positive oligodendrocyte survival in these white matter regions (not significant). Umbilical cord occlusion, or low or high-dose clonidine-hydrochloride infusion in asphyxiated or sham fetuses was not associated with significant changes in numbers of CNPase- and Ki67-positive cells in periventricular and intragyral parasagittal white matter (not significant). The histological analyses for astroglial (GFAP) and microglial expression (Iba-1) in these animals and white matter regions are currently in progress.

Conclusions: These preliminary data suggest that umbilical cord occlusion and low-dose, but not high-dose, clonidine-hydrochloride infusion are independently associated with dramatic loss of total oligodendrocytes, but have no synergic deleterious effect. These findings highlight a complex, region- and dose-specific relationship for catapres, given that low-dose clonidine protected subcortical neuronal structures in the same paradigm.

Long-term effects of low dose dexamethasone and ventilation therapies on alveolar development in preterm lambs

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Background: Preterm infants may develop a chronic respiratory disorder called bronchopulmonary dysplasia (BPD). Contemporary BPD is reportedly characterised by large-simplified alveoli with reduced secondary septation and is associated with prolonged mechanical ventilation (MV). Postnatal steroids may be required to wean infants from MV. However, the long-term impact of contemporary low-dose postnatal steroids on postnatal alveolarisation remains unclear.

Aim: To investigate the effects of ventilation strategy and low dose dexamethasone on postnatal alveolar development in preterm lambs within a contemporary neonatal clinical setting and treatment approach.

Methods: Double-blind randomised controlled trial. Preterm lambs (129 d) were randomised to non-invasive ventilation (NIV) or MV at birth, commencing either postnatal saline (Sal) or low-dose (DART protocol) dexamethasone (DEX) at 72 h: NIV/Sal (n=6); NIV/Dex (n=6); MV/Sal (n=7); MV/Dex (n=5). Term lambs (n=8) (150 d gestation) were used as controls. Lambs were raised to 2 months corrected postnatal age then killed humanely (pentobarbitone 150 mg/kg). Five lung samples were obtained from the left lung and inflation fixed to 30 cm H_2O (10 % formalin). Fixed tissue was embedded, sectioned and stained with hematoxylin and eosin for morphological assessment of lung development through stereological analysis using Visiopharm newCAST Stereology Software.

Results: Lung volume and parenchymal volume were higher in preterm than term lambs ($p<0.05$). However, there were no significant differences in septal volume, alveolar surface area and alveolar number between preterm and term lambs, or consequent to mechanical ventilation or postnatal steroids. The differences observed in lung and parenchymal volumes may be due to hyper-inflation and residual parenchymal thickening.

Conclusions: Preterm lambs managed with contemporary clinical strategies including volume-guarantee ventilation have parenchymal thickening and hyperinflation but no evidence of failed alveolarisation. Ventilation strategy and duration, and use of low-dose postnatal steroids do not affect parenchymal and alveolar structure at 2 months corrected postnatal age in 129 d preterm lambs.

Funding/Support: NHMRC GRT1057514, GRT1057759, RF1077691. Chiesi Farmaceutici S.p.A. (poractant alfa), Fisher & Paykel Healthcare (ventilation circuits), ICU Medical (monitoring lines).

Abstracts – Session 6

Session 6 (Queenstown Room)				
Chairs: A/P Tim Moss and Dr Joanne Davidson				
Time	**No.**	**Speaker**		**Student/ECR**
11:10am	A28	Madison Paton	Assessing the efficacy of human umbilical cord blood versus mesenchymal stem cell therapy for preterm brain inflammation	Late PhD
11:25am	A29	Kyra Chan	Early administration of umbilical cord blood cells increases systemic inflammation and blood-brain barrier breakdown in injuriously ventilated preterm lambs	Early PhD
11:40am	A30	Tayla Penny	Sex differences in long-term behaviour following perinatal brain injury	Early PhD
11:55am	A31	Madeleine Smith	Do umbilical cord blood cells reduce ventilation-induced lung injury in preterm infants?	Honours
12:10pm	A32	Elliot Teo	Human placental-derived stem cells for hypoxic-ischaemic encephalopathy	Early PhD
12:20pm	OPEN		General discussion	
12:35pm to 1:35pm Delegate Lunch (Bazar Restaurant)				

Assessing the efficacy of human umbilical cord blood versus mesenchymal stem cell therapy for preterm brain inflammation

Madison Paton[1,2], Beth Allison[1], Michael Fahey[1,3], Amy Sutherland[1], Robert Bischoff[1],Ilias Nitsos[1], Timothy Moss[1], Graeme Polglase[1], Graham Jenkin[1,2], Courtney McDonald[1]* and Suzanne Miller[1,2]*.

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Background: There are no current therapies that target and protect the preterm brain following in-utero inflammation and cerebral white matter injury. Umbilical cord blood (UCB) and mesenchymal stem cells (MSCs) are widely studied cell therapies for neurological complications. However, their respective efficacies have never been directly compared in the same animal model and laboratory. This study aimed to compare the efficacy of either UCB or MSCs in a large animal model of inflammation-induced preterm brain injury.

Methods: Chronically instrumented fetal sheep at 0.65 gestation were administered I.V. LPS (LPS, 150ng, 055:B5) or saline (control), over 3 consecutive days. Six hours after the final LPS dose, animals received either 100 million human UCB mononuclear cells, or 10 million cord tissue-derived MSCs, I.V.. Seven days later, CSF was collected, and brains processed for histological analysis of the white matter.

Results: LPS administration resulted in increased microglial accumulation (lectin+), astrogliosis (GFAP+), CSF IL1β (p=0.06), cell death (cas-3, p=0.02) and reduced total white matter and mature white matter cells (Olig-2+, p=0.03 and MBP+, p=0.05, respectively) compared to controls. UCB administration resulted in lower CSF IL1β (p=0.02), significantly less cell death (p=0.03) and normal white matter cell maturation compared to LPS alone. MSC treatment reduced CSF IL1β (p=0.09) from LPS alone with significantly less cerebral astrogliosis (p=0.03), but did not prevent the loss of mature white matter cells to the same extent as UCB (Olig-2+ and MBP p>0.9 MSC group versus LPS alone, p<0.2 in UCB group compared to LPS alone).

Conclusions: Both UCB and MSCs protect against LPS-induced neuroinflammation. However, UCB is significantly more effective at restoring normal oligodendrocyte maturation, compared to MSCs. This work demonstrates that consideration is required to determine which cellular therapy is appropriate, particularly when selecting a treatment to reduce preterm white matter injury.

Early administration of umbilical cord blood cells increases systemic inflammation and blood-brain barrier breakdown in injuriously ventilated preterm lambs

Kyra Chan[1,2], Ilias Nitsos[1], Valerie Zahra[1], Domenic LaRosa[1], Vanesa Stojanovska[1], Suzanne Miller[1,2], Dhafer Alahmari[3], Graeme Polglase[1,2], Courtney McDonald[1]

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Background: Initiation of ventilation in the delivery room can cause brain injury in preterm neonates (ventilation-induced brain injury; VIBI) through two main pathways: cerebral inflammation and haemodynamic instability. Umbilical cord blood (UCB) cell therapy is suggested to be neuroprotective by targeting inflammation but its interaction with mechanical ventilation has not been studied.

Aims/Hypothesis: To investigate the effects of early UCB administration on VIBI in preterm lambs.

Methods: Fetal lambs (0.85 gestation) were exteriorised, ventilated with an injurious strategy for 15 min with placental circulation intact, and returned to the uterus. Lambs were randomised to controls (INJ; n=7) or UCB-treated (80 million allogeneic UCB cells in 3 ml PBS via umbilical vein 1 h post-ventilation; INJ+UCB; n=7). At 24 h, lambs were delivered and maintained on non-injurious ventilation for up to 1 h during which lambs underwent magnetic resonance imaging (MRI). Brains were collected after MRI; immunohistochemistry was used to assess inflammation and vascular leakage in the periventricular and subcortical white matter (PVWM; SCWM). Inflammatory cytokine levels in blood plasma collected over 24 h were assessed with ELISA.

Results: No increases in systemic interleukin(IL)-6 and -8 levels were observed in INJ animals over 24 h. IL-6 and IL-8 levels peaked 6 h and 3 h post-ventilation respectively in INJ+UCB animals and were higher than that in INJ animals ($p=0.002$; $p<0.001$). The number of blood vessels in the PVWM with protein extravasation was higher in INJ+UCB compared to INJ lambs ($p=0.004$). The number of microglial aggregations and the density of microglia within aggregations were higher in INJ+UCB than INJ lambs in the SCWM ($p=0.048$; $p=0.007$).

Conclusions: Early administration of UCB cells increased systemic inflammatory cytokine levels at 3 h and 6 h, and protein extravasation in the PVWM of injuriously ventilated lambs. The timing of UCB administration needs to be further investigated. UCB administration within the first few hours of ventilation, coinciding with peak inflammation, may be detrimental and delayed administration may instead be favourable.

Sex differences in long-term behaviour following perinatal brain injury

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Background: Hypoxia ischemia (HI) during labour and birth can result in injury to the brain, termed hypoxic ischemic encephalopathy (HIE) and subsequent long term neurodevelopmental disorders, such as cerebral palsy (CP). Studies have shown that in the clinic, the rates of HIE and CP are higher in males. While it is known that sex differences occur in animal models of perinatal brain injury, no studies have looked at long-term behavioural outcomes, or the impact of sex differences on the outcomes of stem cell treatment.

Aims/Hypothesis: This study examined potential sex differences following HIE in the neonatal rat, and to see if these differences are maintained in the long term and reflected in behavioural outcomes. Additionally, this study explored sex differences in response to treatment with umbilical cord blood (UCB) cells. We hypothesise that males will be impaired in behavioural tests and that UCB administration will ameliorate behavioural deficits in males and females.

Methods: HI injury was induced in postnatal day (PND) 10 rats by left carotid artery ligation, followed by 90min hypoxia (8% oxygen). At postnatal days 11, 13 and 20, pups received 1 million human UCB cells intranasally (total 3 million cells). Rats were monitored until PND50; throughout this period, they underwent extensive behavioural testing including novel object recognition (NOR).

Results: Preliminary results revealed that once corrected for body weight, females had significantly larger brains than males in all groups ($P<0.0001$). Additionally, HI females had significantly smaller brains than the sham females ($p=0.02$), no differences were observed between male groups. The NOR test showed that on PND30 there was a significant sex difference ($p=0.01$), where females had impairment after HI insult, but males did not. UCB treatment in the females appeared to reverse this impairment. At PND50 this sex difference was not observed in the NOR and impairment was evident in both male and female HI groups. UCB reversed this deficit in both sexes.

Conclusions: Our results highlight that long-term sex differences are observed in both brain size and short-term memory deficits. Surprisingly, females had increased deficits compared to males, which was opposite to our hypothesis. Encouragingly, UCB treatment was able to reverse deficits in both males and females.

Do umbilical cord blood cells reduce ventilation-induced lung injury in preterm infants?

Madeleine Smith, Graeme Polglase, Courtney McDonald, Paris Papagianis, Ilias Nitsos, Valerie Zahra, Kyra Chan

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Background: Preterm infants have immature lungs and consequently, many require respiratory support at birth. Unfortunately, respiratory support, is a major cause of BPD. We have previously shown that high tidal volumes cause lung inflammation and injury. Our attempts to reduce this so far have been ineffective. Various therapies have been trialled to reduce lung inflammation and injury, but have shown little benefit. Umbilical cord blood (UCB) contains five known cell types that reduce inflammation and injury and is a promising treatment for reducing lung inflammation and injury.

Aims/Hypothesis: To determine whether UCB cells can reduce lung inflammation and injury following injurious ventilation in preterm lambs.

Methods: Pregnant ewes underwent sterile surgery at 124 ± 1(SD) days gestation. Lambs were exteriorized via caesarean section, injuriously ventilated for 15 minutes, and then returned to the uterus and the ewe allowed to recover. One hour post ventilation, fetuses were randomized to receive 80 million ovine UCB cells (CELLS) or saline (INJ), administered to the fetal jugular vein. Regular blood-gases and plasma were collected. 24 hours later, lambs were delivered for an MRI prior to euthanasia. Lungs were collected for histological and molecular assessment of lung inflammation and injury and compared to lambs that did not receive injurious ventilation (controls).

Results: Histological indices of inflammation and injury, including wall thickness and total injury score, were significantly higher in injuriously ventilated groups compared to controls, but there was no difference between INJ and CELL lambs. Hyaline membrane sloughing and haemorrhage in tissue were not different between groups. The number of CD45 positive cells was not different between CELLS and INJ lambs, but both groups had significantly higher CD45 positive cells compared to controls.

Conclusions: Preliminary histological assessment of the lungs demonstrated UCB cells did not reduce lung inflammation and injury. Further investigations are underway to determine the efficacy of UCBs in reducing other structural and molecular indices of inflammation and injury.

Human placental-derived stem cells for hypoxic-ischaemic encephalopathy

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Background: Neonatal hypoxic-ischemic encephalopathy (HIE) results in a high rate of mortality and severe long-term disability. The current gold-standard treatment of HIE, therapeutic hypothermia, still leaves 1 in 4 babies dead and 1 in 4 survivors with cerebral palsy. There is, therefore, a compelling need for additional therapies. Stem-cell therapy is an attractive candidate due to its immunomodulatory, neurotrophic, angio-, neuro- and synaptogenic properties. My PhD project will evaluate high dose mesenchymal and stromal stem-cells from human placenta for the treatment of neonatal HIE.

Aims: To determine the effectiveness of sorted placental stem cells as an adjunctive neuroprotectant in our pre-clinical large animal model of neonatal HIE.

Methods: Piglets will undergo a moderate hypoxic-ischaemic injury and will receive either stem-cells or saline (vehicle). All animals will undergo hypothermia for 24h, rewarmed and allowed to recover to post-HI day 7. Neurobehavioral testing will occur daily, and MRI and MR/spectroscopy performed on post-HI day 7 prior to euthanasia. Neuropathology will be evaluated by histology, immunohistochemistry and western blotting to assess effects of stem cell therapy particularly in the context of inflammation.

Significance: This study will be the first to provide clear evidence of the effectiveness of high dose sorted, placental stem cell therapy for HIE.

Abstracts – Session 7

Session 7 (Queenstown Room)				
Chairs: Prof Alistair Gunn and Dr Stephanie Millar				
Time	**No.**	**Speaker**		**Student/ECR**
01:35pm	A31	Kelly Zhou	The effect of ischemia and hypothermia on axonal and myelin integrity	Early PhD
01:50pm	A32	Antoniya Georgieva	Computerized analysis of intrapartum electronic fetal monitoring and hypoxic-ischemic encephalopathy at Oxford during 2012-2016	Early career
02:05pm	A34	Nathanael Yates	Ex Vivo MRI Examination of Preterm Lamb Cortical Development Following Postnatal Dexamethasone	Early career
02:20pm	A36	Kate Goasdoue	A time-course of blood-brain barrier disruption in a large animal model of neonatal hypoxic-ischemic encephalopathy	Late PhD
02:35pm	A37	Joanne Davidson	The effect of the rate of rewarming after cerebral hypothermia on post-ischemic white matter injury in the near-term fetal sheep	Not Applicable
03:05pm – 3:35pm Afternoon Lunch (Foyer)				
03:35pm – 04:00pm Prize giving and Farewell				

The effect of ischemia and hypothermia on axonal and myelin integrity

Kelly Zhou[1], Vittoria Draghi[1], Christopher Lear[1], Justin Dean[1], Jesse Ashton[1], Yufeng Hou[1], Laura Bennet[1], Alistair Gunn[1] and Joanne Davidson[1]

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Background: Hypoxic ischemic encephalopathy (HIE) is associated with a high risk of disability. The standard treatment of 72 hours of hypothermia is partially effective, with many infants still suffering disabilities. We have previously shown that the area fraction and integrity of myelin in the intragyral white matter was reduced after cerebral ischemia in near-term fetal sheep, but was restored with hypothermia. It is not known whether this disruption in myelination results from oligodendrocyte loss, or underlying axonal pathology.

Aims/Hypothesis: To assess myelin and axonal integrity after global cerebral ischemia and determine the effect of hypothermia for 48 or 72 hours in near-term fetal sheep.

Methods: Fetal sheep (0.85 g.a.) were randomised to sham control (n=9), ischemia-normothermia (n=8), ischemia-48 h hypothermia (n=8) or ischemia-72 h hypothermia (n=8). Axons were labelled with SMI312 and myelin with MBP, in the intragyral white matter of the first (IGWM1). SMI312 was co-labelled with GFAP (astrocytes) and Iba1 (microglia). Axonal and myelin directionality was analysed with ImageJ plug-in – OrientationJ as a measure of integrity, and axonal morphology was assessed qualitatively.

Results: Ischemia was associated with a 12% loss of axonal linearity and a 12% loss of myelin linearity in IGWM1 compared to sham control ($p<0.05$), but was restored with 48 and 72 hours of hypothermia. In sham controls, axons had a dense, linear appearance. After ischemia, they were finer, sparser, and had a spheroid morphology. This improved with 48 and 72 h of hypothermia, but some abnormal axonal morphologies persisted. The loss of SMI312 staining was colocalised with GFAP and Iba1.

Conclusions: Ischemia was associated with structural abnormalities in both axons and myelin. Both 72 and 48 hours of hypothermia restored axonal and myelin linearity, but the presence of abnormal axonal morphology was only partially attenuated. These suggest that axonal pathology may underlie abnormal myelination, which may contribute to motor deficits seen in infants, even among those treated with hypothermia.

Computerized analysis of intrapartum electronic fetal monitoring and hypoxic-ischemic encephalopathy at Oxford during 2012-2016

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Background: We are developing a system for computerised electronic fetal monitoring (EFM) in labour that aims to ultimately reduce the incidences of both hypoxic-ischemic encephalopathy (HIE) and unnecessary operative deliveries. An early prototype OxSys 1.5 was developed using data from over 23,000 neonates at Oxford born between 2000 and 2011 (Georgieva et al, *Acta Obstet Gynecol Scand* 96(7), 2017).

Aims: To apply OxSys 1.5 to a new cohort of infants with HIE (born in 2012-2016) for whom clinical and neuroimaging data were available and to present an initial analysis of this cohort.

Methods: We reviewed all 85 neonates born in 2012-2016 at the Oxford John Radcliffe Hospital with moderate or severe HIE or who received hypothermia for neuroprotection. Included here were only the 73 infants with digital intrapartum EFM, which was analysed retrospectively with OxSys 1.5 (no impact on clinical practice).

Results: Sixty-eight (93%) underwent hypothermia treatment; 67 (92%) had cord gas analyses: only 21 (31%) with arterial pH<7.0 and 30 (45%) <7.05. OxSys had a sensitivity of 43% for HIE, in line with our results published previously using a composite 'severe compromise' outcome*. This compared to 24 (33%) operative deliveries in clinical practice due to abnormal EFM. Nearly 70% of OxSys alerts were given more than 90min before birth.

Table 1. Frequency of events of interest, all percentages are from the total of 73 infants.

Male	48 (65.8%)	Neonatal death before MRI scan	7 (9.6%)
Abnormal admission ultrasound	4 (5.5%)	Neonatal death after MRI scan	2 (2.7%)
MRI scan (median age: 8 days)	59 (80.8%)	OxSys 1.5 Alerts	31 (43.4%)
- *Normal*	*40 (54.8%)*	- *Alert due to nonreactive initial trace*	*7 (9.6%)*
- *Basal Ganglia injury*	*6 (8.2%)*	- *Alert due to high Decelerative Capacity*	*24 (33%)*
- *White matter injury*	*9 (12.3%)*		
- *Mixed*	*4 (5.5%)*	Operative delivery due to presumed fetal compr.	24 (32.9%)
Thick meconium	19 (26.0%)	Prolapse/rupture/abruption/should. dystocia	19 (26.0%)

Conclusions: The presented cohort constitutes a valuable resource for future developments of OxSys. We will aim to increase substantially its sensitivity for timely detection of the infants at risk of HIE by analysing this data to improve our understanding of the physiological pathways to HIE that can be manifested in the EFM.

Ex Vivo MRI Examination of Preterm Lamb Cortical Development Following Postnatal Dexamethasone

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Background: MRI offers an opportunity to align imaging with histological structure in preclinical settings. However, establishing this link necessitates overcoming challenges in *ex vivo* MRI acquisition and analysis of fixed tissue. We aimed to determine if *ex vivo* MRI on fixed brains could depict changes in cortical development after preterm birth and postnatal dexamethasone – a glucocorticoid associated with impaired neurodevelopment.

Aims/Hypothesis: Aims: 1) Develop MRI protocols and analytical techniques to overcome the unique challenges of *ex vivo* MRI, and 2) determine how postnatal dexamethasone dose affects cortical development.

Methods: Preterm lambs were randomised to three groups with tapering doses of 1) saline (n = 8); 2) low-dose dexamethasone (N = 8) or 3) high-dose dexamethasone (N = 8). Lambs were delivered surgically on day 129. Preterm lambs were managed with standard clinical protocols and euthanised at 7 d. Naive end-point fetal controls were delivered at 136 d gestation (N = 7). *Ex vivo* post-fixed brain halves were imaged with high-resolution 3D MRI using a 9.4 T Bruker BioSpec with a T1 weighted sequence. MRI software analysis utilized the Characterization Virtual Laboratory (M3 MASSIVE, Monash).

Results: Poor contrast on initial tissue imaging improved substantially improved through imaging sequence optimisation, bias field correction (N4ITK), and non-local means de-noising. Semi-automated grey/white matter segmentation was achieved by establishing landmarks for prefrontal cortex segmentation and use of supervised machine learning (iLastik). ANOVAs revealed that postnatal dexamethasone had no effect on prefrontal cortex (PFC) volumes (total, p = 0.97; white matter, p = 0.68; grey matter, p = 0.93) or white/grey matter ratio (p = 0.81).

Conclusions: Our workflow allowed for successful MRI analysis of *ex vivo* preterm lamb brains, consistent with previous histology, which showed no changes in white matter maturation, cortical thickness or cell density. Postnatal dexamethasone did not alter MRI measures of cortical development 7d postnatal age.

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A time-course of blood-brain barrier disruption in a large animal model of neonatal hypoxic-ischemic encephalopathy

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Background: Perinatal hypoxic ischaemic encephalopathy (HIE) is a serious disorder that occurs in 1-5 per 1000 live term births and HIE is associated with increased risk of death and disability. Blood-brain barrier (BBB) disruption is a key mediator of HI injury, however neonatal BBB-disruption remains incompletely characterised. Understanding the time course of BBB-disruption may advance treatments that improve neurodevelopmental outcomes for neonates following HI injury.

Aims/Hypothesis: This project aims to characterise the time-course of BBB-disruption in a large animal model of birth asphyxia.

Methods: Anaethetised, ventilated, neonatal pigs (<24h) were exposed to a HI insult by a reduction in inspired oxygen from 21% to 4% for approximately 30min. Animals (n=4/group) were culled at 2, 4, 8, and 12h post-HI insult. Parietal cortex and hippocampal sections were investigated via immunofluorescence and western blotting for serum IgG extravasation, as well as neurovascular unit alterations (astrocytes, neurons, and microglia). qPCR was also utilised to investigate expression of BBB-associated genes (CLDN5, ZO1, OCLN, AQP4).

Results: BBB-disruption was detected as early as 2h post-HI, determined by IgG extravasation, and alterations to tight-junctions at the BBB. IgG extravasation appears to follow a biphasic pattern – there was much greater permeability of IgG in parietal cortex at 2 and 12h post-HI, but less so at 4 and 8h post-HI. Immunofluorescence demonstrates that microvessels with IgG leakage appear to have lost astrocyte coverage. mRNA expression of tight junction genes at the BBB was altered over time – with CLDN5 mRNA expression increasing, but ZO1, and OCLN decreasing.

Conclusions: This study has demonstrated that BBB-disruption following HI-injury is dynamic and rapid. This has implications in our understanding of other disease processes such as inflammation and excitotoxicity, as well as treatment options as the BBB is traditionally seen as a hindrance to drug delivery.

The effect of the rate of rewarming after cerebral hypothermia on post-ischemic white matter injury in the near-term fetal sheep

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Background: Therapeutic hypothermia partially reduces death and disability in neonatal hypoxic-ischemic encephalopathy. It is widely suggested that rewarming slowly can improve outcomes, but there is limited evidence. We recently showed that after 48 hours of hypothermia, slow rewarming over 24 hours partially improved recovery of EEG power, but not neuronal survival.

Aims/Hypothesis: The aim of this study was to determine the effect of slow rewarming over 24 h after 48 h of mild cerebral hypothermia on white matter injury, after global cerebral ischemia in near-term fetal sheep.

Methods: Fetal sheep (0.85 gestation) received 30 min ischemia followed by normothermia (n=8), or hypothermia from 3 h until 2 days after ischemia, followed by rapid rewarming (ischemia-2d, n=8), or 2 days plus slow rewarming over 24 hours (ischemia-2d+slow, n=8) or 3 d plus rapid rewarming (ischemia-3d, n=8). Fetuses were killed at day 7 for histology.

Results: Ischemia was associated with loss of total and mature oligodendrocytes ($p<0.05$), myelin basic protein (MBP) and CNPase, with increased Iba1-positive microglia and GFAP-positive astrocytes. Numbers of oligodendrocytes were improved by all hypothermia protocols but only ischemia-3d partially attenuated loss of mature, MBP positive, oligodendrocytes. The area fraction of MBP was significantly increased by hypothermia overall, and, in the IGWM1, was restored to sham control values in the ischemia-2d+slow group. The area fraction of CNPase was significantly increased after ischemia-2d+slow compared to ischemia-3d in the PVWM. Iba1-positive microglia were suppressed by hypothermia, with fewer microglia in the ischemia-2d+slow group compared to ischemia-2d in the IGWM1 and PVWM but more than ischemia-3d in the PVWM. Similarly, the increase in GFAP-positive astrocytes was attenuated more in the ischemia-2d+slow group compared to both ischemia-2d and -3d in the IGWM1, than ischemia-2d in IGWM2.

Conclusions: Slow rewarming after hypothermia did not improve oligodendrocyte survival but was associated with apparent improvements in some aspects of inflammation and myelination.

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