Fetal and Neonatal Workshop

Friday 12 – Saturday 13 April 2013

Barossa Valley SA

The Fetal and Neonatal Workshop is affiliated with the Perinatal Society of Australia and New Zealand Annual Scientific meeting.

We thank the Perinatal Society of Australia and New Zealand, and the School of Paediatrics & Reproductive Health for their sponsorship of the 2013 Fetal and Neonatal Workshop:

THE PERINATAL SOCIETY OF AUSTRALIA & NEW ZEALAND

www.psanz.com.au

THE UNIVERSITY of ADELAIDE

<u>2013 FNW POC:</u> Dr Rob De Matteo, Dr Nicolette Hodyl, Dr Kathy Gatford, Dr Beverley Muhlhausler, Dr Michael Stark

PROGRAMME

Speaker names and abstract titles are given below – full details of co-authors are on the abstracts. Speakers with "" next to times are eligible for student awards. Speakers with "E" next to times are eligible for ECR awards.

The index of author names is at the end of the abstract booklet.

THURSDAY APRIL 11th 2013
PRE-CONFERENCE BAROSSA EXPERIENCE - Optional Winery Tour for participants.

DAY 1 FRIDAY APRIL 12th 2013

0900 Registration

1000 Opening words

1015 Cardiovascular and ventilation orals (each 10 + 5 minutes)

1015* #1 Kimberley Botting - Developmental changes in cardiac expression of miR and their targets involved in cardiomyocyte proliferation in fetal and newborn sheep.

1030* #2 Monalisa Padhee - Impact of *in vitro* culture and embryo transfer on cardiovascular function and heart growth in postnatal life.

1045* #3 Paul Lombardo - Ultrasound assessment of the left ventricle, kidneys and common carotid arteries in three month old lambs born moderately preterm.

1100* #4 Vivian Nguyen - The effect of moderate preterm birth on postnatal growth, heart rate and blood pressure

1115 #5 Justin Lang - Partial aeration with 100% nitrogen at birth induces a global increase in pulmonary blood flow

1130 #6 Domenic La Rosa - Maternal dietary creatine supplementation prevents changes in diaphragm muscle function 1 month after asphyxia at birth

1145 PANEL DISCUSSION

1200 Lunch

1300 Brain and behaviour orals (each 10 + 5 minutes)

1300* #7 Karinna Fyfe - Preterm Infants are Vulnerable to Reduced Cerebral Oxygenation in the Prone Position.

1315* #8 Jessica Gugusheff - A maternal 'junk food' diet alters the response of the mesolimbic reward system to naloxone in offspring post-weaning.

1330* #9 James Aridas - Hypothermia in newborn lambs: Keeping it cool.

1345* #10 Damien Hunter - Placental restriction of fetal growth induces sex-specific changes to learning in maze tasks in adolescent and young adult sheep.

1400E #11 Luke Schneider - The role of corticomotor excitability in cognitive outcomes after preterm birth.

1415 #12 Laura Bennet - Sweet is definitely not 'sweet' when it comes to preterm brain injury.

1430 PANEL DISCUSSION

1445 Afternoon tea

1500 DOHaD orals (each 10 + 5 minutes)

1500* #13 Maria Nguyen - Intrauterine inflammation and the early development of atherosclerosis: a mechanistic study using a novel murine model.

1515E #14 Song Zhang - Impact of *in vitro* embryo culture and transfer on adrenal growth and key regulatory genes in the adrenal of six month old lambs.

1530* #15 Amy Wooldridge - Does late pregnancy methyl donor supplementation reverse effects of placental restriction on immune function in the sheep?

1545 #16 Anne Jaquiery - Twin conception results in increased fat mass and altered metabolism in adult sheep.

1600* #17 Jane Pereleshina - Can birth hypoxia affect ovarian follicular reserve?

1615 #18 Ray Rodgers - Studies of fetal origins of polycystic ovary syndrome

Short oral (5 + 3 minutes)

1630 #19 Alison Kent – Can 670NM red light protect against retinopathy of prematurity and reduce lung injury in a neonatal animal model?

PANEL DISCUSSION

1638

1710 End of session

1800 Dinner

DAY 2 SATURDAY APRIL 13th 2013

0900 Preterm birth

Orals (each 10 + 5 minutes)

0900E #20 Shigeo Yamaoka - Pentraxin 3, a locally produced inflammatory marker, in bronchoalveolar lavage fluid predicts the severity of bronchopulmonary dysplasia.

0915E #21 Pauline Decima - Thermal management in closed incubators: new software for optimizing the neonate's thermohygrometric environment.

0930* #22 Erin McGillick - The fetal sheep lung does not respond to glucocorticoids during the late canalicular phase, the current limit of viability for the human infant.

0945 #23 Sarah Robertson - TLR-mediated inflammatory pathways–new insights in pre-term and term birth.

Short orals (each 5 + 3 minutes)

1000* #24 Sheena Bouch - A novel model for retinopathy of prematurity in adult mice exposed to hyperoxic gas after birth.

1008* #25 Natalie Aboustate - Innate immune regulation in the preterm neonate.

1016 #26 Kathy Gatford - Placental restriction impairs glucose homeostasis and its response to hyperglycaemic challenge.

1024E #27 Yvonne Eiby – Angiotensin II, Angiotensin 1-7 and skin blood flow during hypoxia in the preterm piglet.

1032* #28 Robert Galinsky – Intrauterine inflammation alters cardiopulmonary but not cerebral haemodynamics during open endotracheal tube suction in preterm lambs.

PANEL DISCUSSION

1040

1055 Morning tea

1115 Pregnancy complications, long-term effects, mechanisms and interventions

Orals (each 10 + 5 minutes)

1115* #29 Stacey Ellery - Maternal creatine supplementation protects the neonatal spiny mouse following birth asphyxia, but what are the effects on the mother?

1130* #30 Yoga Kandasamy - Extra-uterine renal growth in preterm infants: Oligonephropathy and prematurity.

1145 #31 Kirsty Pringle - Novel urinary biomarkers for predicting pregnancy outcome in indigenous and non-indigenous Australian women.

Short orals (each 5 + 3 minutes)

1200* #32 Mini Vithayathil - Effect of 'Cafeteria diets' on the fatty acid composition of her milk and offspring.

1208* #33 Melinda Dolan - Determining the role of glucocorticoids in inflammation-induced fetal lung maturation using glucocorticoid receptor knockout mice.

1216* #34 Hong Liu – Neonatal exendin-4 normalises glucose tolerance and insulin secretion in IUGR sheep.

1224 #35 Stefan Hiendleder– Widespread differential maternal and paternal genome effects on bovine fetal muscle and bone development at midgestation.

1232E #36 Sean O'Leary – The effect of maternal vitamin D status at 15 weeks gestation on pregnancy outcome.

1240* #37 Kristina Sobotka - The effect of carotid blood flow steal in asphyxiated near-term lambs.

1248* #38 Stacey Hokke - Impaired maternal glucose tolerance alters nephron endowment and impairs renal function in male offspring.

1256 PANEL DISCUSSION

1310 Lunch

1400 Placenta orals (each 10 + 5 minutes)

1400* #39 (Lucky) Sultana Mahabbat-e Khoda - Effects of oxygen on the expression of hypoxia inducible factors (HIFs) in first trimester placenta.

1415* #40 Angela Cumberland - Placental neurosteroidogenesis and neonatal outcome following preterm birth.

1430* #41 Jessica Laurence - Dietary vitamin D and calcium restriction and its effects on murine placental morphometry.

1445* #42 Jing Zhou - Omega-3 fatty acid treatment stimulates proliferation of the placental trophoblast cell line HTR8/SVneo.

1500 #43 Tamas Zakar - Gene expression control by histone modifications in the human amnion.

1515 #44 Vicki Clifton - The human placenta expresses multiple glucocorticoid receptor isoforms and expression is altered by fetal sex and maternal asthma.

1530 PANEL DISCUSSION

1545 Judging, prizes and final words

1600 Close

Developmental changes in cardiac expression of miR and their targets involved in cardiomyocyte proliferation in fetal and newborn sheep

Janna L Morrison[1], Doug A Brooks[2], I Caroline McMillen[1], Song Zhang[1], Enzo R Porrello[3], Kimberley J Botting[1]
[1]Early Origins of Adult Health Research Group, University of South Australia, Australia; [2]Mechanisms in Cell Biology and Diseases Research Group, University of South Australia, Australia; [3]Laboratory for Cardiac Regeneration, School of Biomedical Sciences, University of Queensland, Australia

Introduction: Recent studies in zebrafish and mice implicate microRNAs (miRs) in the regulation of genes responsible for cell cycle progression in the heart, in particular the miR-15 family (miR-15a, -15b, -16, -195 and -497), miR-199a, miR-590 and miR-133. miRs are small non-coding RNAs that regulate the abundance of protein by targeting mRNA for degradation or inhibiting translation. In mice, members of the miR-15 family are upregulated in early postnatal life, corresponding to a time when cardiomyocytes lose the ability to proliferate and regenerate a heart after injury. The timing of cardiomyocyte maturation in mice differs from humans, such that the ability of murine cardiomyocytes to proliferate is lost between 4-10d postnatal life, but the transition to terminally differentiated cardiomyocytes begins in late gestation in humans and in sheep.
Aim/Hypothesis: We aim to test the hypothesis that miRs responsible for the regulation of proliferation will increase with increasing age and the mRNA expression of their target genes will decrease in the fetal and postnatal sheep heart.
Methods: We have measured the cardiac expression of miRs involved in cell cycle entry in the sheep fetus at 90d gestation (when all cardiomyocytes are mononucleated and capable of proliferation; term=150d), 121d gestation (when ~75% of cardiomyocytes are capable of proliferation), 141d gestation (when ~25% of cardiomyocytes are capable of proliferating), 5d and 21d after birth (when almost all cardiomyocytes are terminally differentiated).
Results: Fold change analysis to 90d gestation indicates that miR-497 increases with increasing age and that miR-15a is increased at 21d. The miR-15 family target genes (*Chek1, Cdc2a, Birc5* and *Spag5*) decrease with increasing age. The expression of miR-199a increases with increasing gestation, but returns to the level of expression observed at 90d gestation at 21d postnatal life. Interestingly, miR-590 does not change with increasing age. miR-199a and miR-590 target genes, *HopX* and *Crim1*, do not change with increasing age, however, *Clic5* unexpectedly increases into postnatal life. miR-133 increases with increasing age, but unexpectedly, so too does the mRNA expression of its target genes *Pgam1* and *Connexin43*.
Conclusion: The overall changes in expression of the miR-15 family and their target genes are consistent with the changes observed in mice across the period of proliferative to terminally differentiated cardiomyocytes in early postnatal life. Interestingly, however, miR-199a, -590 and -133 and their target genes are not consistent with the timing of changes in their expression in mice. This suggests that proliferation of sheep cardiomyocytes is subtly different to that of mice, but suggests that the sheep heart may be capable of regeneration during fetal life.

Impact of *in vitro* culture and embryo transfer on cardiovascular function and heart growth in postnatal life

Monalisa Padhee[1], I Caroline McMillen[1], Severence M MacLaughlin[1], Song Zhang[1], David O Kleemann[2], Simon K Walker[2], Janna L Morrison[1]

1 Sansom Institute for Health Research, University of South Australia, Adelaide, South Australia, 5000.
2 Turretfield Research Centre, South Australian Research and Development Institute, Adelaide, South, Australia, Australia.

Padmy002@mymail.unisa.edu.au

Background: Nutrition during the periconceptional period is critical in determining cardiovascular health in postnatal life. In this study, we aimed to determine if *in vitro* embryo culture and transfer, manipulations to the nutritional environment during the periconceptional period, change cardiovascular function and heart growth in postnatal life.

Methods: Embryos were either transferred to an intermediate ewe (ET) or cultured *in vitro* in the absence (IVC) or presence of human serum (IVCHS) and a methyl donor (IVCHS+M) for 6d. Naturally mated (NM) ewes acted as controls. At 22 wks, basal blood pressure recordings were carried out before phenylephrine (intravenous; 4,8,16 and 20 µg/kg) was administered. At 24wks, hearts were collected and mRNA expression of receptors and markers of hypertrophy were measured.

Results: There was no difference in basal blood pressure and a significant inverse relationship between mean arterial pressure and heart rate in NM, ET and IVCHS in response to phenylephrine stimulation. However, this relationship was blunted in the IVC and IVCHS+M groups. There was no difference in gene expression of receptors (IGF1R, IGF2R and αAR1A) but an increase in ETAR in IVCHS+M females only. There was no change in gene expression of markers of hypertrophy, such as ANP and MHCβ.

Conclusions: This study demonstrates that although there is no change in basal blood pressure or major signalling molecules regulating cardiac growth, the blunting of baroflex sensitivity in offspring exposed to IVC and IVCHS+M and increase in ETAR gene expression in IVCHS+M indicates compromised cardiovascular function which may result in increased risk of hypertension in later life.

Ultrasound assessment of the left ventricle, kidneys and common carotid arteries in three month old lambs born moderately preterm.

Lombardo P[1],Schneider ME[1], De Matteo R[2], Harding R[2], Nguyen V[2], *Polglase GR[3] and *Black MJ[2].
[1]Department of Medical Imaging & Radiation Sciences, Monash University, Clayton, Australia. [2]Department of Anatomy & Developmental Biology, Monash University, Clayton, Australia. [3]The Ritchie Centre, Monash Institute of Medical Research, Clayton, Australia. *Joint senior authors. Email: paul.lombardo@monash.edu

Background: Structural changes to the heart, vasculature and kidneys in response to preterm birth may lead to an increased risk of cardiovascular disease in later life. We conducted longitudinal *in vivo* ultrasound analyses in preterm and term lambs three months after birth to examine growth of the heart, blood vessels and kidneys.
Method: Moderately preterm birth (0.9 of term) was induced (Epostane) in seventeen Border Leicester x Merino ewes after antenatal corticosteroids (Celestone Chronodose: 11.4 mg i.m.; 2 doses, 24hrs apart). Eight female and nine male lambs were born vaginally at 131±1 days gestational age (dGA). A control group of lambs was induced to be born at term (147±1 days dGA) and comprised six female and eleven males. Ultrasound imaging was conducted on all lambs at approximately three months (13±1 weeks) after birth to measure the structure and function of the left ventricle, kidney dimensions and renal artery blood flow velocities, as well as the diameter and blood flow velocities within the aortic root, pulmonary arteries and the right and left common carotid arteries. Three measurements of each parameter were obtained and averaged, then corrected for body weight and compared using a two-way ANOVA and Tukey post-hoc analysis of preterm/term and male/female factors and their interaction.
Results: At three months of age, preterm lambs weighed less than term lambs (15.8%, $p=0.002$). Left ventricular hypertrophy was evident in the hearts of preterm lambs, with an increase in the anterior (16.5%, $p<0.05$) and posterior (14.2%, $p=0.003$) wall systolic thickness of the left ventricle and in the systolic (15.7%, $p=0.011$) and diastolic (12.2%, $p=0.034$) thickness of the interventricular septum. The percentage fractional shortening of the cardiac muscle was also increased (12.7%, $p=0.011$) and the aortic root diameter was enlarged (11.1%, $p=0.043$) in the preterm lambs. Systolic blood flow velocity in the aortic root was increased (17.8%, $p=0.037$) and the diameter of the right (16.1%, $p=0.009$) and left (13.9%, $p=0.044$) common carotid arteries were increased in the preterm lambs compared to term lambs. The right kidney length (10.7%, $p<0.05$) and width (9.7%, $p=0.012$) dimensions were also increased compared to term lambs. The left kidney could not be demonstrated adequately using ultrasound. There were no significant sex differences found in any of the comparisons.
Conclusion: Lambs born moderately preterm exhibit a thicker left ventricular wall, increased left ventricular contraction and larger common carotid vessel diameter than term lambs when adjusted for body weight at three months of age. These findings are likely to be an adaptive response to preterm birth and/or glucocorticoid administration. The relationship between these findings and subsequent cardiovascular vulnerability into adulthood will be examined.

The effect of moderate preterm birth on postnatal growth, heart rate and blood pressure

Vivian Nguyen[1], Robert De Matteo[1], Richard Harding[1], *Graeme Polglase[2] & *M Jane Black[1]

[1]Dept of Anatomy & Developmental Biology, Monash University, VIC 3800, Australia,
[2]The Ritchie Centre, Monash Institute of Medical Research, VIC 3168, Australia.
*Joint senior authors
vivian.nguyen@monash.edu

Background: Preterm birth affects 8% of all live births in Australia, with the majority of these born moderately preterm. Mothers at risk of preterm birth are often administered antenatal corticosteroids to help with lung maturation. Epidemiological studies have demonstrated that there is an increase in arterial pressure in adults who have been born preterm; this may relate to changes in body growth that occur after birth or due to antenatal corticosteroid treatment.
Aims/Hypothesis: This longitudinal study aimed to compare postnatal growth, arterial pressure and heart rate in sheep born moderately preterm with those born at term.
Methods: Using a well-established ovine model of moderately preterm birth, Border-Leicester-Merino cross ewes carrying singletons were induced (Epostane; 50mg in 2ml EtOH i.v.) to deliver vaginally at 0.9 of term (131±1days of gestation, n=17) or term (147±1days of gestation, n=17). For survival of preterm lambs, clinically relevant doses of Betamethasone (11.4mg, i.m.) were also given 24 and 48 hours before birth. After birth, measurements of body weight, heart rate, arterial pressure, crown-rump length, thoracic girth and limb lengths were taken weekly for 12 weeks then monthly afterwards. For statistical analysis: 3 way ANOVA; factors: sex, gestational type and postnatal age.
Results: At birth, preterm lambs were lighter than term controls (4.21±0.13kg vs 6.44±0.28kg, $p<0.0001$). After birth, body weight of preterm lambs increased by 1.56±0.03kg/week, whereas term controls increased by 1.85±0.04kg/week, indicating the growth rate in term lambs was 18% greater than in preterm lambs. Overall, males were larger than females ($p=0.007$) during the study period. All measured body dimensions were significantly smaller in preterm lambs. There was a significant overall reduction ($p<0.0001$) in mean arterial pressure after birth in preterm lambs; however, no difference in heart rate was observed.
Conclusions: During the first 5 postnatal months, preterm lambs were smaller and lighter than term controls and this was accompanied by a reduction in arterial blood pressure. Continued monitoring of these lambs for 12 months will determine whether these differences persist into adulthood.

Partial aeration with 100% nitrogen at birth induces a global increase in pulmonary blood flow

Justin Lang[1], James Pearson[2], Megan Wallace[1], Melissa Siew[1], Marcus Kitchen[3], Andreas Fouras[4], Kevin Wheeler[5], Graeme Polglase[1], Arjan te Pas[6], Stuart Hooper[1]

[1]The Ritchie Centre, MIMR, VIC 3168, Australia, [2]Monash Biomedical Imaging, VIC 3168, Australia, [3]School of Physics, Monash University, VIC 3800, Australia, [4]Division of Mechanical and Aerospace Engineering, Monash University, VIC 3800, Australia, [5]Royal Hobart Hospital, TAS 7000, Australia, [6]Dept. Pediatrics, Leiden University, 2333 CC Leiden, Netherlands.
Justin.Lang@monash.edu

Background: Aeration of the lungs at birth increases pulmonary blood flow (PBF). The mechanisms behind the increase in PBF are thought to be the entry of air and removal of lung liquid, and exposure of the pulmonary microcirculation to an increase in oxygen (O_2) levels. Our recent studies demonstrated that aeration of the right lung resulted in increased PBF in both lungs; that is in aerated and non-aerated regions (Fig.1). This surprising result has led to this study investigating the relative effects of O_2 on the increase in PBF at birth.
Aims/Hypothesis: We aimed to determine how partial ventilation without O_2 (using 100% nitrogen (N_2)) affects the PBF changes at birth using simultaneous phase contrast (PC) X-ray imaging and angiography. It was expected that an absence of O_2 would not prevent the increase in PBF in unventilated regions but attenuate the increase in PBF in ventilated regions when compared to ventilation with air carried out in an earlier study.
Methods: Newborn rabbits (n=6) were delivered near-term (~30 d GA; term ~32 d GA) and immediately PC X-ray imaged while an iodinated contrast agent was infused into the jugular vein, to visualise PBF before and then during aeration of one lung with 100% N_2 gas. The inspired gas was switched to air (21% O_2), and both lungs were then aerated. The number of visible pulmonary blood vessels, their diameter and the relative change in intensity was measured, the latter providing a relative measure of PBF.
Results: Aeration of one lung with 100% N_2 increased visible vessel number (from 32±2 to 52±8), and percentage change from background intensity (from 11.9±4.4 % to 25.2±2.4 %) in the liquid-filled and unaerated lung. All measured variables were not different between aerated and non-aerated lung regions (Fig.1). All variables increased after a switch to air ventilation ($p<0.05$), and increased after both lungs were ventilated ($p<0.05$).
Conclusions: Partial lung aeration promotes a global increase in PBF, resulting in a significant ventilation/perfusion mismatch in unventilated lung regions, despite an absence of oxygen. This study demonstrates that mechanisms other than O_2 concentration are responsible for the increase in PBF at birth.

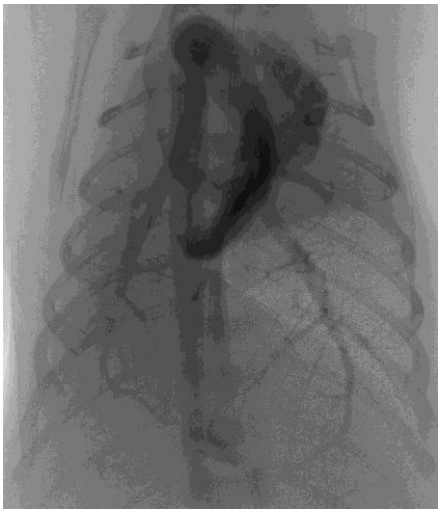

Fig.1. PC X-ray Image of a newborn rabbit ~30 seconds after aeration of the right lung (with 100% N_2) and immediately after a bolus of iodine has passed through the heart and entered the lungs.

Maternal dietary creatine supplementation prevents changes in diaphragm muscle function 1 month after asphyxia at birth.

Domenic A LaRosa[1], Stacey J Ellery[1], Helena C Parkington[2], Rod J Snow[3] David W Walker[1] & Hayley Dickinson[1].

[1]Ritchie Centre, Monash Institute of Medical Research, Monash University, VIC 3800, Australia; [2]Department of Physiology, Monash University, Victoria 3800 Australia; [3]Centre for Physical Activity and Nutrition, Deakin University, Burwood, Victoria 3125.
domenic.larosa@monash.edu

Background: Birth asphyxia occurs in 4/1,000 live term births and if prolonged can result in energy failure, tissue damage, and even fetal death. Using our established model of near-term birth asphyxia in the spiny mouse [1], we have shown that intrapartum hypoxia for 7.5 minutes causes 40% pup mortality, and at 24 hours of age and, in surviving animals, significant brain, kidney and muscle damage, and reduced contractile force in the diaphragm. Maternal dietary creatine (Cr) supplementation from mid-pregnancy improves neonatal survival by >20% and protects the brain, kidney and diaphragm from hypoxia-induced damage. However, the long-term effects of birth asphyxia and Cr supplementation on the diaphragm musculature are not known.
Aims/Hypothesis: This study assessed: (i) the effects of birth asphyxia on the diaphragm muscle at 33 days of age; and (ii) the role of Cr in protecting this important respiratory muscle against hypoxic damage at birth. We hypothesized that birth asphyxia would result in structural and functional changes in the diaphragm that persist, and that Cr would either prevent or minimize these changes.
Methods: Pregnant spiny mice were fed a control or 5% Cr-supplemented diet from day 20 of gestation (term ~39 days). On day 38 of pregnancy, fetuses underwent either an asphyxic or c-section birth as previously described [1]. Animals were then cross-fostered to a lactating dam for 33 days ± 2 days. At post-mortem a portion of the diaphragm muscle was used for an *in vitro* study of twitch tension and fatigue using field stimulation via external electrodes.
Results: Pups from the birth asphyxia group showed significantly reduced postnatal growth compared to c-section controls, whereas pups from the birth asphyxia + Cr group were not different to controls. Functional analysis of the diaphragm revealed no differences in peak twitch tension or twitch contraction and relaxation times between any of the groups. Muscle fatigue, induced by a train of pulses (1x330ms train/sec, 400Hz for 5 mins), was significantly greater for birth asphyxia pups compared to controls, whereas the muscle fatigue from birth asphyxia + Cr was not different from control values. Maximum tetanic force was significantly reduced in the birth asphyxia group, but not in the birth asphyxia + Cr group.
Conclusions: Maternal dietary Cr supplementation prevents asphyxia-induced delay in postnatal growth, and ameliorates the reduction in maximum tetanic force and increased fatigue in diaphragm muscle that follows birth asphyxia. These results show that birth asphyxia produces long-lasting deficits in diaphragm muscle function but importantly, that these are largely prevented if Cr is supplemented via the maternal diet before birth.

1. Ireland Z, Dickinson H, Snow R, Walker DW. Maternal creatine: does it reach the fetus and improve survival after an acute hypoxic episode in the spiny mouse *(Acomys cahirinus)*? Am J Obstet Gynecol 2008;198:431.e1-431.e6.

Preterm infants are vulnerable to reduced cerebral oxygenation in the prone position.

Karinna Fyfe[1], Sasha Odoi[1], Stephanie Yiallourou[1], Flora Wong[1,2,3], Adrian Walker[1], Rosemary Horne[1]

1. The Ritchie Centre, Monash Institute of Medical Research, Melbourne.
2. Monash Newborn, Monash Medical Centre, Melbourne.
3. The Department of Paediatrics, Monash University, Melbourne.

Karinna.Fyfe@monash.edu

Background: Preterm infants are at greater risk of the Sudden Infant Death Syndrome (SIDS) than term-born infants. SIDS is hypothesised to occur due to an uncompensated hypotensive episode combined with a failure to arouse from sleep, with infants most at risk at 2-3 months of age. We have previously shown cerebral oxygenation (TOI) to be reduced in the prone position, a major risk factor for SIDS, in term infants. Preliminary data suggests TOI is also reduced in preterm infants slept prone. **Hypothesis and Aims**: We hypothesised that preterm infants have a greater fall in TOI in the prone position than term infants. We aimed to determine TOI in preterm infants in both prone and supine sleep, and compare with term infants. **Methods**: 22 preterm (27-36 weeks gestation (GA)) and 17 term infants (39-42 weeks GA) underwent daytime polysomnography (PSG) at 2-3 months post-term age. In addition to the standard PSG measures, TOI was recorded using near-infrared spectroscopy (NIRO-200 spectrophotometer, Hamamatsu Photonics KK, Toyko, Japan) and blood pressure (BP) was measured using a Finometer (Finometer^{TM}, Finometer Medical Systems, Amsterdam, The Netherlands). Infants were slept in both the prone and supine positions and in both active (AS) and quiet sleep (QS). Baseline TOI and the percentage TOI difference between the supine and prone positions calculated for each infant. The effect of preterm birth was determined using two-way ANOVA. **Results**: Preterm infants exhibit significantly lower TOI when compared to term infants, in both AS and QS when prone and in AS when supine ($P< 0.05$). Furthermore, there is a strong trend for preterm infants to display a greater delta TOI from supine to prone than term born infants in both QS (preterm: -9.26; term: -4.27; $P=0.068$) and AS (preterm: -8.53; term: -2.06; $P=0.096$). This is despite no difference in the delta BP from supine to prone between preterm and term infants. **Conclusion:** Prone sleeping is associated with a fall in TOI in both term and preterm infants. This fall tends to be greater in preterm compared to term infants, most prominently at 2-3 months post-term age, the period of greatest SIDS risk. Although the reason for the TOI change remains unclear, we suggest this fall in TOI may leave preterm infants vulnerable to critically impaired cerebral TOI and contribute to their increased risk for SIDS.

A maternal 'junk food' diet alters the response of the mesolimbic reward system to naloxone in offspring post-weaning.

JR Gugusheff[1], ZY Ong[1, 2] and BS Muhlhausler[1, 2]

[1]Foodplus Research Centre, School of Agriculture, Food and Wine, The University of Adelaide, South Australia, Australia.[2] Sansom Institute for Health Research, School of Pharmacy and Medical Science, University of South Australia, South Australia, Australia.

jessica.gugusheff@adelaide.edu.au

Background: The consumption of highly palatable but nutrient poor 'junk foods' in western society is escalating and thus determining what mediates an increased preference for these foods has become of growing importance. We have shown that offspring of mothers who consume a junk food diet during pregnancy and lactation not only consume more junk food as adults[1] but also have a desensitized opioid pathway[2], which in part mediates reward response in the brain. The present study aims to further explore the mechanisms behind the desensitization of the opioid pathway in the offspring of junk food fed mothers by exploring alterations in gene expression in response to early life blockade of opioid receptors.

Aims/Hypothesis: To determine whether the response to the opioid antagonist naloxone in rat offspring was influenced by exposure to a junk food diet in the perinatal period

Methods: 17 Albino Wistar female rats were provided with either a junk food (JF, n=9), or control chow diet (C, n=8), during pregnancy and lactation. At weaning, pups received daily intraperitoneal injections of either naloxone (5mg/kg) or an equivalent volume of saline for 10 days. qRT-PCR was used to determine the expression of mu-opioid receptor (MOR) and the opioid enkephalin in the nucleus accumbens (NAc) and ventral tegmental area (VTA) in the brains of offspring.

Results: In male offspring, there was a significant interaction ($P<0.05$) between maternal diet and saline/naloxone treatment on MOR expression in the VTA, such that MOR expression was decreased by naloxone treatment in the JF group, but not controls. In the NAc, MOR expression in males (C 0.003±0.0007, JF 0.006±0.0008, $P<0.05$) and enkephalin expression in females (C 1.29±0.04, JF 1.43±0.04, $P<0.05$) was increased in JF offspring independent of naloxone treatment

Conclusions: These results indicate that perinatal exposure to a junk food diet alters the response of the mesolimbic reward pathway to opioid receptor blockade. This outcome together with our previous work showing that the opioid antagonist naloxone was less effective at reducing palatable food intake in the offspring of junk food fed dams provides evidence that dysregulation of the opioid pathway may be responsible for the increased preference for junk food observed in offspring exposed to a junk food diet in early life.

[1] Ong Z, Muhlhausler B (2011) Maternal "junk-food" feeding of rat dams alters food choices and development of the mesolimbic reward pathway in the offspring *The FASEB Journal*.

[2] Gugusheff JR, Ong ZY, Muhlhausler BS (2013) A maternal "junk-food" diet reduces sensitivity to the opioid antagonist naloxone in offspring postweaning *The FASEB Journal* **27**, 1275-84.

Hypothermia in newborn lambs: Keeping it cool

James Aridas[1], Tamara Yawno[1], Jingang Li[1], Amy Sutherland[1], Michael Fahey[1,2], Atul Mulhotra[1,2], Flora Wong[1,2], Alistair Gunn[3], Euan Wallace[1,2], Graham Jenkin[1] &, Suzie Miller[1].

[1]The Ritchie Centre, Monash Institute of Medical Research, VIC 3168, Australia

[2]Monash Medical Centre, VIC 3168, Australia

[3]Department of Physiology, University of Auckland, Auckland 1142, New Zealand

james.aridas@monash.edu

Background: Hypoxic ischaemic encephalopathy (HIE) describes the clinical syndrome following a severe lack of oxygen during labour. HIE is associated with permanent neurological impairment and death. Hypothermia treatment has been a great advance in the care of newborns with HIE; meta-analysis of RCTs indicate that hypothermia reduces long-term morbidity and death in childhood. Despite the efficacy of hypothermia, nearly 50% of children still suffer permanent neurological impairment. Only one in nine cases of cerebral palsy, a permanent neurological movement disorder, is prevented with adequate cooling therapy. These modest improvements dictate the need for adjuvant therapies to improve the efficacy of hypothermia.

Aims/Hypothesis: Hypothermia has not previously been undertaken in a term newborn lamb model of hypoxic ischaemic encephalopathy. With this study we aimed to develop a newborn lamb model of hypothermia that allows us to develop adjuvant neuroprotective treatments.

Methods: HIE was induced via umbilical cord occlusion (UCO) at caesarean section in near-term lambs (141 days). Lambs were resuscitated, stabilised and monitored after delivery. Whole body hypothermia commenced 4 hours after delivery and was achieved with cooling bags of crushed ice surrounding lambs. Core temperature was assessed vie rectal temperature probe. The initial protocol (see below) was associated with an unsatisfactory mortality rate. Alterations to the protocol were able to improve this figure.

Protocol	Cooling begun	Length of cooling	Temperature	12h MRI	72h MRI	Cardiovascular support
Initial	**4h**	**72h**	**34°C**	**YES**	**YES**	**NO**
	4h	72h	35°C	NO	YES	NO
	4h	48h	35°C	NO	YES	NO
Current	**4h**	**24h**	**35°C**	**NO**	**YES**	**YES**

Results: We have found cooling to be associated with a gradual decrease in blood pressure over hours. Cardiovascular support with fluid resuscitation, inotropes, chronotropes, and steroids have shown mixed success in maintaining blood pressure. Altering the length of cooling and temperature is able to improve survival rate.

Conclusions: Hypothermia therapy is the key treatment strategy for newborns suffering hypoxic ischaemic encephalopathy but 50% of babies still suffer permanent damage. Newborn lambs are unable to adequately maintain cardiovascular and respiratory control when undergoing the hypothermia protocol used in human babies. So that we can investigate adjuvant treatments, we are modifying the protocol to further elucidate treatment options to improve the effectiveness of hypothermia.

Placental restriction of fetal growth induces sex-specific changes to learning in maze tasks in adolescent and young adult sheep

Damien Hunter[1,2,3], Kathryn L Gatford[1,2], Karen L Kind[1,3], Hong Liu[1,2], Miles De Blasio[1,2], Julie A Owens[1,2], Julia Pitcher[1,2], Susan Hazel[1,3]

[1] Robinson Institute, [2]School of Paediatrics & Reproductive Health and [3]School of Animal and Veterinary Sciences, University of Adelaide

Email: damien.hunter@adelaide.edu.au

Background: Intrauterine growth restriction (IUGR) retards neurodevelopment, particularly of the hippocampus [1]. IUGR children have impaired hippocampal-associated cognition, including learning and short term memory [2]. In humans, both deficient and excess cortisol levels impair learning [3]. IUGR has sex-specific effects on the stress axis in children. Low birth weight boys secrete more cortisol when stressed than those of normal birth weight [4], which may also contribute to cognitive impairment. However, examining these mechanisms, and the interactions between the two, is difficult in humans due to postnatal confounding factors such as socioeconomic status. We therefore investigated effects of restriction of placental and fetal growth (PR) in sheep on cognition utilizing a maze protocol.

Hypotheses: 1. Restriction of placental and fetal growth (PR) will impair learning speed, cognitive flexibility and short term memory in adolescent and young adult sheep. 2. PR will impair these outcomes to a greater extent in males than in females.

Method: PR was induced in Border Leicester x Merino ewes by removal of the majority of endometrial placental implantation sites ≥10 weeks before mating. Maze testing was performed at 18 and 40 weeks of age in control (CON, n = 16M, 14F) and PR progeny (n = 6M, 7F). Learning, memory, cognitive flexibility, and bleat frequency (a behavioural stress measure), were examined by sequential training, learning and reversal tasks. Effects of treatment, sex, age and task were analysed by repeated measures ANOVA.

Results: The effects of PR on learning measures and behavioural stress differed by sex. PR males took more trials to solve each task than CON males, whereas PR females took fewer trials than CON females (sex*treatment, $p=0.018$). When adjusted for animal speed (time for each task relative to time taken on the initial training task), 18 wk CON females took less time than PR females to learn reversal and retention tasks, related to learning extinction and memory respectively, whereas PR males took longer than CON males (sex*treatment, $p<0.0001$). At 18 weeks, PR males bleated more frequently than CON males, and PR females less than CON females (sex*treatment, $p=0.045$). Inclusion of bleat frequency as a covariate in analysis of maze performance removed the effects of PR on relative time to learn each task, but the effects of PR on the number of trials remained.

Conclusions: Consistent with our hypothesis, effects of PR on learning speed and cognitive flexibility differed with sex, with males but not females being impaired. Differences in apparent stress appear to account for some but not all of these sex-specific effects of PR. The greater relative time the PR males took during reversal tasks suggests poorer cognitive flexibility, whereas the more rapid learning extinction in females may indicate a cognitive advantage due to decreased stress following PR.

1. Lodygensky, G. et al., Pediatr Res.2008; 63(4): 438-443.
2. Pylipow, M. et al., J Pediatr. 2009; 154(2):201-6.
3. Schilling, TM. et al., Psychoneuroendocrinology. 2013; 13: S0306-4530
4. Jones, A., J Clin Endocrinol Metab. 2006; 91(5): 1868-1871.

The role of corticomotor excitability in cognitive outcomes after preterm birth.

Luke A. Schneider[1], Nick R. Burns[2], Ted J. Nettelbeck[2], Michael C. Ridding[1], & Julia B. Pitcher[1].

[1] Robinson Institute, School of Paediatrics and Reproductive Health, University of Adelaide, SA 5005, Australia, [2] School of Psychology, University of Adelaide, SA 5005, Australia.

luke.schneider@adelaide.edu.au

Background: A number of cognitive domains have been shown to be impaired by preterm birth (Baron & Rey-Casserly, 2010), but the underlying pathophysiology associated with these deficits is not clear. However, cerebral white matter (WM) is particularly sensitive to damage following preterm birth (Nagy *et al.*, 2003), which in turn is increasingly implicated in cognitive outcomes (Soria-Pastor *et al.*, 2008; Tuch *et al*., 2005). The resting motor threshold (rMT) to transcranial magnetic stimulation (TMS) reflects motor cortex excitability and underlying WM integrity and maturation (Klöppel *et al.*, 2008) and may therefore provide a useful correlate of WM integrity/maturation in studies of cognitive ability and gestation in children.

Aims/Hypothesis: The main aim of the study was to investigate the relationships between gestational age at birth (GA), corticomotor excitability (i.e. rMT), and cognitive abilities in children born 24-41 weeks GA. The influences of several social, maternal and perinatal factors on cognitive outcomes were also investigated.

Methods: 145 children (78 boys) born 24-41 weeks GA were administered the Woodcock-Johnson III - Tests of Cognitive Abilities (Woodcock *et al.*, 2001) at age 12.4 years ± 0.8 months. Single pulse TMS over the hand area of the primary motor cortex and surface electromyography was used to obtain rMT for left and right index finger muscles. Obstetric and neonatal information was obtained with consent from medical records and each child's postnatal community health "blue book".

Results: GA negatively correlated with rMT in both hemispheres ($r = -0.31$, $p \leq 0.0001$). Higher general intellectual ability scores were associated with greater GA ($r = 0.17$, $p \leq 0.05$) and lower rMT ($r = -0.25$, $p \leq 0.01$). 'Early preterm' children exhibited significantly poorer working memory performance than 'late preterm' or 'term' children ($p < 0.05$). Other cognitive abilities negatively associated with rMT included verbal ability ($p < 0.05$), thinking ability ($p < 0.01$), and phonemic awareness ($p < 0.05$). Cognitive efficiency was negatively associated with rMT ($p < 0.05$) and positively associated with GA ($p < 0.05$). Other predictors of cognitive outcomes were identified by relative importance regression modelling and included sex, birthweight centile, birth length and head circumference, parity, socioeconomic status, and child height at the time of cognitive assessment.

Conclusions: Cortical excitability (i.e. rMT) appears a better predictor of cognitive outcome than GA per se, suggesting that the motor cortex provides a useful sentinel marker of overall cortical development. The lack of relationship between GA and performance on several cognitive tasks suggests that factors affecting post-natal cortical development, particularly socioeconomic disadvantage at birth, have important influences on cognitive outcomes in children. Taken together, these findings suggest that while preterm birth increases the risk of poor cortical development and hence, suboptimal cognitive outcomes, this can be modulated be several postnatal factors.

Sweet is definitely not 'sweet' when it comes to preterm brain injury.

Rachael Robson, Chris Lear, Joanne Davidson, Sam Mathai, Alistair Jan Gunn, Laura Bennet.
Department of Physiology, The University of Auckland, Auckland, New Zealand.
Email: l.bennet@auckland.ac.nz

Background: Hypoglycaemia is associated with adverse neural outcomes in some preterm infants. However, the range of low plasma glucose concentrations that could potentially result in brain injury remain undefined. Paradoxically, however, elevated glucose levels are associated with both reduced and increased brain injury when administered in conjunction with hypoxia-ischemia (HI). Thus safe levels of perinatal plasma glucose concentrations in association with injurious insults remain poorly understood.

Aims/hypothesis: The aim of this study was to examine the role of glucose in modulating brain injury when elevated before an HI insult in preterm fetal sheep.

Methods: 5 ewes carrying preterm fetal sheep at 0.7gestation were given an intramuscular injection of dexamethasone (12mg) 4 h before an HI insult of 25 min of umbilical cord occlusion (UCO). In a separate group, fetuses received an infusion of glucose to elevate fetal plasma glucose to either 2.5 mmol/l (vs control 1.1mmol/l, n=2) or 3mmol/l (n=2), starting 4 h pre-UCO. Control fetuses (n=5) received a vehicle infusion starting 4 h pre-UCO. All fetuses were studied for 5 days after UCO.

Results: Histological assessment of the dexamethasone group showed that 2/5 fetuses developed significant cystic periventricular leucomalacia (PVL), and subcortical and cortical injury ($P<0.001$). The remaining 3 fetuses had significantly greater white matter and subcortical neuronal injury, but no cystic lesions (vs. controls. $P<0.001$). PVL was associated with fetal plasma glucose levels of 2.8 and 3.1mmol/l at the start of UCO. The same findings of PVL were observed in glucose infusion fetuses who plasma glucose levels above 3mmol/l. Fetal measures of EEG, impedance, nuchal EMG and heart rate suggest PVL started to evolve early post-HI.

Conclusions: Our preliminary results show that elevated fetal plasma glucose levels are associated with increased neural injury after severe UCO, with levels above 2.8 mmol/l associated with PVL. The data suggest that it is not the duration of exposure to glucose, but the plasma glucose concentration at the time of HI which is important. Further studies are required to determine the glucose concentration range which may mediate increased injury in association with different types of perinatal insults and treatments.

Intrauterine inflammation and the early development of atherosclerosis: a mechanistic study using a novel murine model

Maria Nguyen[1], Megan Wallace[1,2], Trevelyan Menheniott[3], Salvatore Pepe[3,4], Tracey Gaspari[5], Robert Widdop[5], David Burgner[3,4]*, Tim Moss[1,2]*

[1]The Ritchie Centre, Monash Institute of Medical Research, [2]Department of Obstetrics and Gynaecology, and [5]Department of Pharmacology, Monash University, VIC 3800, Australia, [3]Murdoch Childrens Research Institute, Royal Childrens Hospital, VIC 3052, Australia, [4]Department of Paediatrics, University of Melbourne, VIC 3010, Australia. *equal senior authors
maria.nguyen@monash.edu

Background: Atherosclerosis is a chronic inflammatory condition that can start during fetal life, particularly in the presence of maternal factors that act through common inflammatory pathways. We hypothesise that intrauterine inflammation (chorioamnionitis) may lead to accelerated development of early atherosclerosis, a concept that has previously been unexplored. As inflammation has been reported to alter epigenetic marks such as methylation, with changes already observed in atherosclerotic lesions, we developed a mouse model of chorioamnionitis to explore such changes.

Aims: *Using a novel model of chorioamnionitis in an atherosclerosis-prone mouse, we aim to investigate:*

1. the effects of intrauterine inflammation on the development of early atherosclerosis in mice
2. potential molecular/epigenetic mediators of atherosclerosis

Methods: Apolipoprotein E deficient (*ApoE-/-*) pregnant mice received intra-amniotic injection of LPS (0.1 ng solubilised in 5 µl saline) or saline (5 µl) into each amniotic sac on gestational day 15 (e15.5). Aortic and heart tissue, and plasma were collected from e18.5, 6-week-old and 12-week-old offspring. Aortae were analysed using quantitative histology following Oil Red O staining and F4/80 antibody immunohistochemistry. Cholesterol concentrations in heart and aortic tissue were quantified using colorimetric/enzymatic assay. mRNA levels of candidate genes were measured using quantitative Real Time-PCR, and DNA methylation studies were performed using the MassARRAY® EpiTYPER Assay (Sequenom).

Results: Intrauterine inflammation significantly increases total cholesterol and cholesteryl ester concentrations in aorta and heart tissue of 6-week-old *ApoE-/-* mice, and appears to increase lipid accumulation and macrophage infiltration in aortic sections. Intrauterine inflammation significantly increases *Dnmt1* mRNA expression, a key gene in the regulation of DNA methylation.

Conclusions: These preliminary data indicate that exposure to inflammation *in utero* may accelerate the development of atherosclerosis and increase *Dnmt1* mRNA expression, suggesting differential epigenetic regulation may be an underlying mechanism.

Impact of *in vitro* embryo culture and transfer on adrenal growth and key regulatory genes in the adrenal of six month old lambs

Song Zhang[1], Janna L Morrison[1], David O Kleemann[2], Simon K Walker[2], I Caroline McMillen[1]

[1]School of Pharmacy and Medical Sciences, Sansom Institute of Health Research, University of South Australia, Adelaide South Australia, 5001. [2]South Australian Research and Development Institute, Turretfield
song.zhang@unisa.edu.au

Background: *In vitro* embryo culture in the presence of human serum in ruminants results in a delay in the timing of parturition. However, it is not known whether *in vitro* embryo culture exerts an effect on adrenal growth and key regulatory genes in the adrenal of lambs in adolescence.
Aims/Hypothesis: We aimed to investigate the impact of *in vitro* embryo culture and transfer on adrenal growth and key regulatory genes in the adrenal of six month old lambs.
Methods: Embryos were collected 24h after artificial insemination of superovulated donor ewes, and were either transferred to an intermediate ewe (ET) or cultured *in vitro* in the absence (IVC) or presence of human serum without (IVCHS) or with methyl donor supplements (IVCHS+M) for six days before transfer to recipient ewes. Naturally mated (NM) ewes were used as controls. Lambs were humanely killed at 24wks of age and adrenal glands were collected, weighed and snap frozen.
Results: The timing of parturition was significantly delayed (P<0.05) in the IVCHS (151.09±0.59d) and IVCHS+M (151.36±0.51d) groups compared with the ET group (149.36±0.49d). Total adrenal weight was lower (P<0.05) in the IVC, IVCHS and IVCHS+M groups than the NM group. Adrenal IGF2R mRNA expression was lower (P<0.05) in the IVCHS and IVCHS+M groups than the ET group. In female but not male lambs, adrenal 3βHSD mRNA expression was lower (P<0.05) in the ET, IVC, IVCHS and IVCHS+M groups than the NM group.
Conclusions: These data suggest that ex vivo manipulation of the sheep embryo results in altered adrenal growth, IGF expression and steroidogenic capacity. Repressed adrenal growth and adrenal IGF2R expression may play a role in the determination of the timing of birth in the *in vitro* embryo cultured groups.

Does late pregnancy methyl donor supplementation reverse effects of placental restriction on immune function in the sheep?

Amy Wooldridge[1,2], Rob Bischof[4], Els Meeusen[4], Hong Liu[1,2], Gary Heinemann[1,2], Damien Hunter[1,2,3], Karen Kind[1,3], Julie Owens[1,2], Vicki Clifton[1,2] & Kathryn Gatford[1,2]. [1]Robinson Institute, [2]School of Paediatrics & Reproductive Health, [3]School of Animal & Veterinary Sciences, University of Adelaide, SA 5000, Australia, [4]Biotechnology Research Laboratories, Dept of Physiology, Monash University, VIC 3800, Australia. amy.wooldridge@adelaide.edu.au

Background: Methyl supplementation during pregnancy increases methyl donors available to the fetus, reducing the risk of neonates developing neural tube defects [3]. However, epidemiological evidence in humans, and from one study in mice, suggests methyl donor supplements in late pregnancy increase the risk of allergy in progeny [1; 4]. Conversely, fetal growth restriction, which reduces methyl donor supplies to the fetus [2], decreases allergy incidence later in life, and we have shown that placental-restriction of fetal growth (PR) decreases allergic responses in sheep.
Aims/Hypothesis: We hypothesised that maternal methyl supplementation in late pregnancy would normalise allergic responses in the adolescent PR sheep.
Methods: Outcomes were measured in 42 control (CON) lambs, 28 PR lambs and 25 PR lambs whose mothers were fed methyl donors (PR+METHYL; 2 g rumen-protected methionine, 300 mg folate, 1.2 g S, 0.7 mg Co/day) from d120 of pregnancy until delivery at term. We measured circulating immune cell populations, antibody response to clostridial vaccination and antibody and skin wheal responses to immunological sensitisation with house dust mite (HDM) and ovalbumin (OVA).
Results: Birth weight was higher in CON (5.2 ± 0.2 kg) than in PR (4.7 ± 0.2 kg, P=0.049) or PR+METHYL lambs (4.2 ± 0.2 kg, P<0.001). Treatment did not affect circulating immune cell populations, antibody responses to clostridial vaccine, or IgE reponses to OVA. PR increased IgE responses to HDM in singletons (P=0.006) and overall (P=0.032), and IgE responses in PR+METHYL sheep did not differ from CON or PR. In singletons, fewer PR than CON sheep had positive skin wheal responses to OVA (P=0.011), with a similar trend overall (P=0.099). The proportion of sheep with positive skin wheal responses to HDM did not differ between treatments.
Conclusions: The decreased skin responses to allergens in PR sheep, despite greater IgE responses, suggest that PR acts downstream of IgE-allergen interactions, possibly via reduced mast cell function. Methyl supplementation in late pregnancy partially reverses this phenotype, consistent with epigenetic mechanisms.

- Hollingsworth JW, Maruoka S*, et al.* (2008) *The Journal of Clinical Investigation* **118**(10), 3462-9.
- MacLennan NK, James SJ, Melnyk S, Piroozi A, Jernigan S, Hsu JL, Janke SM, Pham TD, Lane RH (2004) *Physiological Genomics* **18**, 43-50.
- Medical Research Council Vitamin Study Research Group (1991) *Lancet* **338**, 131-7.
- Whitrow MJ, Moore VM, Rumbold AR, Davies MJ (2009) *American Journal of Epidemiology* **170**(12), 1486-93.

Twin conception results in increased fat mass and altered metabolism in adult sheep

[1,2,3]Anne Jaquiery, [1]Danielle Smeitink, [1,3]Mark Oliver, [1]Serina Hancock, [1]Clare McLean, [1,2,3]Frank Bloomfield
a.jaquiery@auckland.ac.nz
[1]Liggins Institute, University of Auckland, NZ
[2]Department of Paediatrics: Child and Youth Health, University of Auckland, NZ
[3]Gravida: National Centre for Growth and Development, NZ

Background: Previous studies in sheep have shown that being conceived as a twin alters birth weight and postnatal growth trajectories and results in increased % fat mass in young adulthood, even if one fetus was removed in early gestation. This suggests that, in twins, early growth and body composition outcomes are largely determined in the periconceptional period. Whether these changes affect later health and metabolism needs to be determined.
Hypothesis: That the effects of twin conception on growth and development will be amplified with age, resulting in altered body composition and deranged metabolism in mature adulthood.
Methods: Twin pregnancies in sheep were randomised to reduction of one twin on day 42 of a 148 d pregnancy by intra-thoracic KCl (Reductions, n=30) or a sham procedure (Twins, n=19). Singleton-bearing ewes also underwent a sham procedure (n=23). Ewes lambed spontaneously. A cohort of the offspring (Twins n=11, Reductions n=10, Singletons n=9) was retained and studied at 3 years of age. Body composition was assessed by dual x-ray absorptiometry (DXA). Glucose tolerance was assessed by intravenous glucose tolerance test and insulin sensitivity by an hyperinsulinaemic euglycameic clamp technique.
Results: Body weight was greater in males than females in all groups, but not different between groups in either sex. In males, Twins and Reductions had greater % fat mass and less % lean mass than Singletons (% Fat mass: Twins 15±2%, Reductions 19±2%, Singletons 8±2%, group effect $p<0.0001$; Lean mass: Twins 63±2%, Reductions 61±2%, Singletons 68±2%; group effect $p=0.001$). Body composition was not different between groups in females. Glucose tolerance was not different between sexes, or between groups in either sex. Males were more insulin sensitive than females in all groups (sex effect $p=0.001$). Twin males tended to be less insulin sensitive than Singletons, with Reductions intermediate (Insulin Sensitivity: Twins 3.7±0.3, Reductions 4.6±0.3, Singletons 5.1±0.5mg.ml.μU^{-1}.kg-1.min^{-1}; group effect $p=0.06$). Insulin sensitivity followed a similar pattern in females, but results were not statistically significant (Twins 2.9±0.4, Reductions 2.7±0.4, Singletons 3.9±0.6mg.ml.$\mu U^{-1}.kg^{-1}.min^{-1}$). There was no independent effect of birth weight or early growth velocity on adult glucose tolerance or insulin sensitivity.
Conclusions: In sheep, the periconceptional event of being conceived a twin has sex specific effects on body composition and metabolism, independent of birth weight or postnatal growth velocity, that persist into mid adult life. Events occurring in the crucial periconceptional period have more profound effects on growth and metabolism in males than females.

Can birth hypoxia affect ovarian follicular reserve?

Evgenia Pereleshina[1,2*], Domenic LaRosa[2], Stacey Ellery[2], Rod Snow[2], David Walker[2], Sally Catt[1], Peter Temple-Smith[1*] and Hayley Dickinson[2*]

[1]Education Program in Reproduction and Development, Department of Obstetrics and Gynaecology, Monash University, Clayton Victoria 3168 Australia; [2]Ritchie Centre, Monash Institute of Medical Research, 27-31 Wright Street, Clayton VIC 3168 Australia

*these authors contributed equally

evgenia.pereleshina@monash.edu

Background: Hypoxia-ischemia is a multifactorial event that occurs in about 4 per 1000 live human births and 4-8% of hypoxic infants die at birth. Many of those who survive experience severe multi-organ damage to the brain, kidneys, heart and lungs. Despite the possibility that birth hypoxia may also cause lasting damage to the ovary, its effects on the mammalian ovary have not been examined previously.

Aims/Hypothesis: We hypothesized that near-term hypoxia would have negative effects on ovarian reserve and development. The aim was to quantify and compare the numbers of different follicle types between the normal and hypoxic prepubertal spiny mouse ovary.

Methods: We used our established model of global, near-term (-1 day term), hypoxia in the spiny mouse. In hypoxia treated pups uteri were isolated for ~8 min in a warm saline bath before C-section. Controls were immediately delivered by C-section. Offspring were cross-fostered to a lactating dam until 33-35days postnatal age. Ovaries were fixed (Bouin's), embedded (glycomethacrylate), serially sectioned (10u) and stained (H&E). Ovarian volume, total follicle number and mean diameter (primordial, primary, secondary, antral) were estimated stereologically using the optical dissector method and were compared between the control (n=3) and the treated (n=8) groups.

Results: The mean number of primordial (3178±288 [mean±SE] vs 2177±334, $p<0.05$) and primordial plus primary (3762±401 vs 2502±271, $p<0.05$) follicles in the control group ovaries was significantly greater than in the hypoxia group (One-way ANOVA, Tukey post-hoc test). Hypoxic ovaries were significantly smaller than controls in volume (0.70±0.21 vs 1.06±0.09, $p<0.02$) and mean primordial follicle diameter (20.99±0.45 vs 22.03±0.15, $p<0.05$). A pilot study that compared the maturation potential of oocytes from the follicles of control and treated ovaries (postnatal D33-35) showed no significant difference in survival or maturation rates *in vitro.*

Conclusions: A brief perinatal exposure of spiny mouse fetuses to hypoxia resulted in a significant reduction in ovarian volume and depletion of the ovarian follicular reserve at postnatal day 33-35 compared with controls. To our knowledge, this is the first study to demonstrate that birth hypoxia causes detrimental changes to mammalian ovaries exposed to hypoxia at birth. The spiny mouse is a proven model for near-term hypoxia and our results raise the possibility that hypoxia may also affect the human ovary which warrants investigation.

Studies of fetal origins of polycystic ovary syndrome

Nicholas Hatzirodos, Katja Hummitzsch & Ray Rodgers.

Robinson Institute, School Paediatrics and Reproductive Health, University of Adelaide, SA, 5005, Australia.
ray.rodgers@adelaide.edu.au

Background: Polycystic ovary syndrome (PCOS) is a common disorder afflicting women of reproductive age. Apart from infertility and hirsuitism the women are at increased risk of diabetes and dyslipidaemia. In our quest to understand the aetiology of PCOS we discovered that a PCOS candidate gene was expressed in ovarian stroma (Hatzirodos et al, 2011) as the stroma penetrated the ovarian primordium from the mesonephros during ovary development (Hummitzsch et al., 2013. This candidate gene, fibrillin 3, regulates TGFβ activity. In stroma in general TGFβ stimulates fibroblast replication and collagen deposition. The PCOS ovary has increased stroma and stromal collagen, suggesting that the action of fibrillin 3 in fetal life could alter the ovary and increase the predisposition to PCOS. However, all animal models of PCOS involve treatments with androgens either of the mothers (rhesus monkey, sheep) during gestation or the newborns of species (rats and mice) where ovary development continues for some weeks after birth.
Aims/Hypothesis: We therefore sought to examine any relationships between TGFβ and androgen action in fetal ovaries.
Methods: We collected bovine ovaries during gestation and conducted and quantitated gene expression by RT-PCR.
Results: We examined the genes encoding the fibrillin 3 and Hic-5 which is known as the *androgen receptor co-activator 55 kDa protein* and as *TGFβ1 induced transcript 1*. We also measured the androgen receptor. We found that as the expression of fibrillin 3 declined at the end of the first trimester expression of Hic-5 and AR increased into the second trimester.
Conclusions: Androgen action on the fetal ovary occurs downstream of fibrillin 3, and its activity could be modulated by Hic-5 which is a TGFβ inducible cofactor for the androgen receptor.

References:

Hatzirodos N, Bayne RA, Irving-Rodgers HF, Hummitzsch K, Sabatier L, Lee S, Bonner W, Gibson MA, Rainey WR, Carr BR, Mason HD, Reinhardt DP, Anderson RA, Rodgers RJ (2011) Linkage of regulators of TGFβ activity in the fetal ovary to polycystic ovary syndrome. *FASEB Journal* 25, 2256-2265.

Hummitzsch K, Irving-Rodgers HF, Hatzirodos N, Bonner W, Sabatier L, Reinhardt DP, Sado Y, Ninomiya Y, Wilhelm D, Rodgers RJ (2013) A new model of development of the mammalian ovary and follicles. PLOS ONE 8(2):e55578.

Can 670NM red light protect against retinopathy of prematurity and reduce lung injury in a neonatal animal model?

Riccardo Natoli[1,2,3], Krisztina Valter[1,2,3], Marconi Barbosa[1,2], Jane Dahlstrom[3,4], Jan Provis[1,2,3] and Alison Kent[3,5]

[1]ARC Centre of Excellence in Vision Science, Australian National University, Canberra, ACT, Australia

[2]Research School of Biological Sciences, Australian National University, Canberra, ACT, Australia

[3]Australian National University Medical School, Canberra, ACT, Australia

[4]Department of Anatomical Pathology, ACT Pathology, Canberra Hospital, Woden, ACT, Australia

[5]Department of Neonatology, Canberra Hospital, Woden, ACT, Australia

Alison.Kent@act.gov.au

Background: Retinopathy of prematurity is a vasoproliferative disorder that can cause blindness and adverse visual outcomes in extremely premature neonates.

Aims: To determine whether 670nm light promotes normal retinal vessel development in a mouse model of Oxygen Induced Retinopathy of Prematurity (OIR) and whether it affects organ development and growth.

Methods: Four groups of C57BL/6J mice were used: 1) Control; 2) OIR - 75% oxygen p7-12 days and normoxia p12-17 days; 3) OIR and 670nm light – 9 J/cm^2 daily from p7-17; 4) 670nm light – 9 J/cm^2 daily from p7-17. At p17 animals were sacrificed and retinal vasculature labelled with Lectin. Neovascularisation and vaso-obliteration were analysed using established protocols. Weight and length measurements were taken daily until the animals were sacrificed. All organs were harvested, weighed and examined macro- and microscopically.

Results: Neovascularisation was significantly reduced in the 670nm treated OIR group ($P < 0.05$). The 670nm treated mice had increased body weight from p13 but no change in length. The OIR+670nm mice had reduced alveolar haemorrhage in comparison to the OIR only mice ($p<0.05$).

Conclusions: Exposure to 670nm red light appears to promote normal retinal vessel development and may protect against ROP. 670nm treatment may also reduce oxygen induced lung injury.

Pentraxin 3, a locally produced inflammatory marker, in bronchoalveolar lavage fluid predicts the severity of bronchopulmonary dysplasia.

Shigeo Yamaoka*, Tohru Ogihara, Shinya Oue, Masashi Hasegawa, Masako Yasui, Tadashi Inatomi, Jun Shinohara, and Hiroshi Tamai.
*Department of Neonatal Medicine and Pediatrics, Osaka Medical College, 2-7 Daigakumachi, Takatsuki, Osaka, Japan.
ped081@poh.osaka-med.ac.jp

Background: Pentraxin 3 (PTX3) is a member of pentraxin superfamily proteins which include C-reactive protein (CRP). Pentraxins are also recognized as acute-phase proteins which are produced in response to inflammatory insults. CRP is produced by hepatocytes mainly in response to IL-6, whereas PTX3 is produced by many kinds of cells in response to various inflammatory stimuli including exposure of pro-inflammatory cytokines such as IL-1β and TNF-α but not IL-6. Furthermore, PTX3 might increase in local tissue more than pro-inflammatory cytokines because pro-inflammatory cytokines can also induce PTX3 production in addition to local inflammatory insults. Thus, PTX3 might reflect local tissue inflammation more sensitively than other inflammatory markers.
Aims : To study the relationship between bronchopulmonary dysplasia (BPD) and local inflammation in lungs by measuring PTX3 and pro-inflammatory cytokines in bronchoalveolar lavage fluids (BALF).
Methods: BALF were taken from ventilated preterm infants ＜ 28 weeks' gestation at ＜72 hours, 1 week (wk), 2wks, 3wks and 4wks of age. PTX3, IL-1β, and TNF-α in BALF were measured by ELISA kits. According to the NICHD definition, infants were divided into the no/mild BPD or moderate/severe BPD group, and the values were compared between the 2 groups.
Results: At 1 and 2wks, BALF PTX3 level was significantly higher in moderate/severe BPD group (n=23) compared with that in no/mild BPD group (n=23) (1wk; 9.8±10.3ng/ml v.s. 4.4±4.9ng/ml, p=0.02, 2wks; 17.0±13.4ng/ml v.s. 5.0±3.4ng/ml, p=0.01). Although a similar trend was seen about BALF IL-1β and TNF-α, those differences were not significant. Multivariate analysis showed that higher levels of BALF PTX3 measured at 1 and 2wks indicated more severely developing BPD after controlling for the other risk factors (gestational age, birth weight, and ventilation days). Among moderate/severe BPD group, BALF PTX3, IL-1β, and TNF-α all declined at 3wks after a marked rise at 2wks, whereas those among no/mild BPD group showed a sustained, gentle elevation within 4wks.
Conclusions: BALF PTX3 can be a good predictive marker for more severely developing BPD (1wk; AUC=0.73, p=0.02, 2wks; AUC=0.75, p＜0.001). BALF inflammatory markers declined suddenly at 3wks among the more severe BPD infants. This result might suggest that an excessive airway inflammation by 2wks leads to a drastic suppression of inflammation later.

Thermal management in closed incubators: new software for optimizing the neonate's thermohygrometric environment.

Décima P.[1], Stéphan-Blanchard E.[1], Delanaud S.[1], Dégrugilliers L.[1,2], Tourneux P.[1,2], Léké A.[1,2] , Bach V.[1], Libert J-P.[1].

[1] PériTox (EA 4285-UMI 01 INERIS), Jules Verne University of Picardy (Amiens, France)
[2] Neonatal and Intensive Care Unit, Amiens University Medical Center (Amiens, France)
E-mail address : pauline.decima@monash.edu

Background: In low-birth-weight (LBW) neonates, transcutaneous water loss is one of the most important thermal exchanges with the environment. In clinical practice, to reduce this body heat loss, the relative air humidity (RH) of the incubator air is often increased to ensure homeothermia and body growth. However, this adjustment is challenging because the impact of elevated RH on the thermoneutral air temperature (T_a), at which LBW neonates should be nursed, has never been well quantified.
Aim: The aim of the study is to provide to medical staff an ease way to set thermoneutral air temperature in closed incubators in function of the configured air relative humidity.
Methods: We used a novel software package (PRETHERM®) to model the neonate's body heat losses in a closed incubator. The software is based on mathematical equations which assess all the body heat transfers with the environment. It takes into account of birthweight, postnatal age (PNA), the clothing thermal insulation and the shape of the incubator. It can estimate the impact of a change in RH on T_a. The software was clinically validated during the first 11 days of life (PNA0-10) of 23 children (gestational age: 29.9 ± 1.2 weeks; birthweight: 1263 ± 292 g) undergoing intensive care. We assessed the impact on T_a of a 20% increase and a 20% decrease in RH for neonates. For diapered neonates, T_a, calculated by the software, was related to birthweight, PNA and RH using multiple regression analysis to enable easy calculation of T_a in clinical situations.
Results: A 20% increase and a 20% decrease in the RH were simulated and impacted T_a , i.e. we found that the impact on T_a was greatest the first five days of life: $-1.66°C<\Delta T_a<1.87°C$ for neonates weighing 1000 g. For diapered neonates weighing between 500 g and 2000 g, in their first four days of life, equations is: $T_a = 43.485 - 0.094\ RH - 0.461\ PNA - 0.001$ body mass ($r^2 = 0.956$; $p<0.001$), whereas the expression between PNA5 and PNA10 is: $T_a = 38.790 - 0.035\ RH - 0.207\ PNA - 0.001$ body mass ($r^2 = 0.853$; $p<0.001$).
Conclusions: Our software quantifies the impact of a change in RH on the thermoneutral temperature for LBW neonates. It constitutes a decision-support tool for patient care and may also serve as a teaching/training aid.

The fetal sheep lung does not respond to glucocorticoids during the late canalicular phase, the current limit of viability for the human infant.

Erin V. McGillick[1,2], Sandra Orgeig[2], I. Caroline McMillen[1], and Janna L. Morrison[1]

Early Origins of Adult Health Research Group[1] and Molecular & Evolutionary Physiology of the Lung Laboratory[2], Sansom Institute for Health Research, School of Pharmacy and Medical Sciences, University of South Australia.
erin.mcgillick@mymail.unisa.edu.au

Background: Currently, antenatal glucocorticoids are prescribed to women at risk of preterm birth to promote fetal lung and surfactant system maturation and support air breathing after birth. In recent years, advances in perinatal medicine have lowered the age of viability of preterm infants to around 24 wks gestation (term = 40 wks), when the lung is in the late canalicular phase of development; however, the minimum age at which the lung is able to respond effectively to glucocorticoid (GC) administration has not been evaluated.

Aim: To evaluate the ability of the very preterm sheep fetus (equivalent to ~23-24 weeks gestation in humans) to respond to cortisol administration during the late canalicular phase of lung development, to influence expression of genes involved in GC availability, lung liquid reabsorption and surfactant system maturation, and compared this to the level of expression in the late gestation fetal lung.

Methods: Vascular catheters were implanted in the ewe and fetus. At 109-116d gestation (term 150 ± 3d), either cortisol (Solucortef, 2-3 mg in 4.4 ml saline/24h; n=9) or saline (n=8) was infused intravenously into the fetus. Late gestation fetuses received saline infusion from 130-140d gestation (n=12). Expression of GC regulatory genes (11βHSD-1, 11βHSD-2 and GC receptor), genes regulating lung liquid reabsorption (amiloride-sensitive epithelial sodium channel (ENAC) -α, -β and -γ subunits; sodium potassium active transport pump (Na K ATPase) subunits, α1 and β1; aquaporin (AQP) -1, -3, -4 and -5) and surfactant proteins (SP-A, -B, -C and -D) in the fetal lung were measured by qRT-PCR. Immunohistochemistry was used to evaluate the numerical density of SP-B positive cells in the alveolar epithelium of fixed lung tissue samples. Data were analysed using a one-way ANOVA with Duncan Post Hoc test ($P<0.05$ was considered statistically significant).

Results: Cortisol infusion had no impact on mRNA expression of the GC receptor or 11βHSD-2, however, there was increased expression of 11βHSD-1. Cortisol had no effect on the expression of genes involved in lung sodium (ENAC α, β, or γ and Na-K-ATPase-β1 subunits) and water (AQP 1, 3 and 5) reabsorption when compared to the level of expression near term. Furthermore, in comparison to the late gestation fetus, cortisol infusion did not increase lung mRNA expression of surfactant proteins (SP-A, -B, and -C) or the number of SP-B positive alveolar epithelial cells.

Conclusions: These data suggest that there is a potential limit at which the lung is able to respond to GCs and that for infants born at the limits of viability, when the lung is in the canalicular phase of development, GC administration may not be effective as a prophylactic measure to promote lung maturation and reduce the risk of respiratory distress syndrome compared to infants born closer to term.

TLR-mediated inflammatory pathways–new insights in pre-term and term birth

Sarah A. Robertson[1], Camilla Dorian[1] and Mark Hutchinson[1].
[1]Robinson Institute and School of Paediatrics and Reproductive Health, University of Adelaide, Adelaide SA 5005, AUSTRALIA.
sarah.robertson@adelaide.edu.au

Background: Premature birth following preterm labour is a common and critical health issue in fetal-maternal medicine with long-term consequences especially for early preterm neonates. The pathophysiology is poorly understood and the causal factors uncertain, but inflammatory mechanisms are clearly implicated.

Aims/Hypothesis: This project seeks to investigate (1) the role of Toll-like receptor 4 (TLR4)-induced inflammation in the physiological process of on-time term delivery; (2) whether activation of TLR4 by bacterial LPS leads to preterm delivery, and (3) whether inhibition of this pathway using small molecule inhibitors of TLR4 signalling may prevent the parturition cascade.

Methods: Mice with genetic deficiency in TLR4 (*Tlr4* null mice) or the MyD88 signalling adaptor (*Myd88* null mice) were used to examine the requirement for TLR4 and MYD88 in term labour and for susceptibility to LPS-induced preterm delivery (PTD). Expression of inflammation genes implicated in parturition was quantified in gestational tissues by qPCR. The effect of administration of TLR4 inhibitor in preventing LPS-induced PTD was investigated in C57Bl/6 (B6) and Balb/c wild-type mice, as well as mice with genetic deficiency in IL10 (*Il10* null mice), which we have previously shown to be highly susceptible to PTD.

Results: A significant delay was seen in the time of parturition in *Tlr4* but not *Myd88* null mutant mice, indicating TLR4 is necessary for the normal progression of labour. This was associated with diminished expression of *Tnfa*, *Il6, Il12* and other inflammation-associated genes in placental and decidual tissue in late gestation. Both *Tlr4* null and *Myd88* null mice were resistant to LPS-induced PTD. LPS-induced PTD was successfully alleviated using TLR4 inhibitors in *Il10* null and B6 mice, preventing fetal loss associated with death in utero and/or early delivery, resulting in on-time birth with normal perinatal characteristics and survival rates in pups. TLR4 inhibitors did not reverse the reduced fetal weight evident in late gestation after LPS administration. However progeny born after exposure to TLR4 inhibitor in late gestation, with or without concurrent LPS administration, showed no substantial adverse effects on growth trajectory (from birth to 20 weeks), body morphometry or health in adulthood. TLR4 inhibitors were less effective in alleviating LPS-induced PTD in Balb/c mice, suggesting strain-specific differences in underlying pathways.

Conclusions: These results indicate that TLR4-activated inflammation is required for normal on-time labour, and is a key trigger for infection-associated preterm labour. This implies a central role for endogenous TLR4 ligands, likely including danger associated molecular patterns (DAMPs) released from gestational tissues, in activating the physiological inflammatory cascade of labour. In the event of infection, LPS-induced inflammation acting via TLR4 causes precocious activation of the inflammatory response. Early intervention with TLR4 inhibitors can inhibit progression of this cascade and prevent preterm birth without adverse perinatal or postnatal consequences in mice. The TLR pathway warrants further investigation as a potential target for new prevention or treatment options in women with threatened PTD.

A novel model for retinopathy of prematurity in adult mice exposed to hyperoxic gas after birth

Sheena Bouch[1], Xiangting Chen[2], Foula Sozo[1], Richard Harding[1], Paul McMenamin[2]
[1]Respiratory Development and Programming Group, Department of Anatomy and Developmental Biology, Monash University, VIC 3800, Australia
[2]Ocular Immunology Group, Department of Anatomy and Developmental Biology, Monash University, VIC 3800, Australia
sheena.bouch@monash.edu

Introduction: Retinopathy of prematurity (ROP) is a significant cause of morbidity in very preterm infants. In Australia, it affects 9.6% of infants born before 32 weeks of gestation who weigh less than 1500g. Mouse models are commonly utilised to determine the underlying mechanisms and disease processes of ROP, including the role of hyperoxic gas exposure. However, previous murine studies on ROP have largely focused on the short-term effects of neonatal hyperoxic gas exposure on the developing eye.

Aim: The aim of this study is to determine the long-term effects of neonatal hyperoxic gas exposure on the development of retinopathy in mice using live *in vivo* imaging and histological analysis.

Method: Neonatal mice (C57BL/6J) were raised in either 40% or 65% O_2 (hyperoxia groups; HYP) from birth until postnatal day 7 (P7d) and then raised in room air until early adulthood (P56d) or middle-age (10 months; P10m). Controls (CON) breathed room air for the duration of the experiment (P56d: CON n=11, 40% HYP n=20, 65% HYP n=10; P10m: CON n=15, 65% HYP n=15). At P56d and P10m, brightfield and *in vivo* fluorescent angiographic imaging of the fundus was performed with a Micron III camera under anaesthesia with subsequent recovery. At autopsy (P56d and P10m), eyes were collected for histological examination and whole-mount retinal immunohistochemistry.

Results: *In vivo* imaging of the fundus indicated that at P56d the 40% and 65% HYP groups and at P10m the 65% HYP group had a persistent hyaloid vasculature with an increased number of branches, multiple large retinal lesions and abnormal retinal vasculature compared to the CON groups. The pathology appeared to be oxygen concentration dependent, whereby the 65% HYP group appeared to have more severe pathology compared to the 40% HYP group at P56d. Histological examination of the 65% HYP group at P56d revealed thinning of the peripheral retina, multiple retinal folds, presence of pseudorosettes in the retina, and presence of hyaloid vessels in the vitreous extending to the posterior lens surface.

Discussion: These preliminary data demonstrate for the first time that neonatal exposure to hyperoxic gas results in oxygen concentration dependent pathology and long-term retinal injury, which is similar to the pathology observed in very preterm infants with ROP. In addition, the use of *in vivo* fundal imaging enabled us to monitor and evaluate the condition of the eye without having to sacrifice the animal; this feature is extremely beneficial in evaluating the effectiveness of possible treatments for ROP in the future. Further studies are required to understand the underlying mechanisms contributing to the persistence of hyaloid vessels which would normally regress around P7d-P13d.

Innate immune regulation in the preterm neonate

Natalie Aboustate[1], Michael Stark[1], Julia Pitcher[1], Vicki Clifton[1], & Nicolette Hodyl[1]
[1]Robinson Institute, School of Paediatrics and Reproductive Health, University of Adelaide, Adelaide, SA 5000.

natalie.aboustate@adelaide.edu.au

Background:
Preterm neonates are at a greater risk, than term neonates, of developing inflammatory related conditions during the neonatal period, such as sepsis and nocosomial infections. Deficiencies in the innate immune response may be an important factor contributing to these poor outcomes. Our preliminary data indicates cord blood collected from preterm neonates produces proportionally higher levels of Interleukin-6 (IL6) following stimulation with Toll-like Receptor (TLR) ligands than term cord blood. My PhD will explore factors that contribute to this excessive pro-inflammatory response.
Emerging evidence demonstrates an important role for microRNAs (miRs) in tightly regulating cellular processes, including inflammation. miRs are known to negatively regulate the inflammatory response by suppressing the transcription of pro-inflammatory molecules at critical points during infection. Certain miRs have been shown to regulate the TLR pathway through targeting the expression of receptor complexes, signalling molecules and cytokines. Currently, little is known of the temporal dynamics, tissue or age-specific effects of miR-related regulation of innate immune responses in term or preterm neonates.
Aims/ Hypothesis:
My PhD aims to identify factors that contribute to an excessive inflammatory response in preterm neonates, specifically focusing on key miRs that regulate TLR signalling. This will be assessed with respect to gestational age and across the neonatal period. We hypothesise that preterm neonates have a dysregulated inflammatory response due to differential miR expression at various stages of gestational development.
Methods:
The response to activation of different TLRs (i.e. TLR2, TLR3 and TLR4) will be assessed in blood collected at delivery and across the neonatal period. miR arrays will be used to assess differential miR expression following stimulation with TLR ligands and candidate miRs will be confirmed using qRT-PCR. Downstream targets of candidate miRs will also be assessed with qRT-PCR and ELISA.
Conclusions:
This study will help determine differences in innate immune function and regulation between term and preterm neonates through identifying whether TLR-stimulated miR expression is related to gestational age and whether it matures across the neonatal period.

Placental restriction impairs glucose homeostasis and its response to hyperglycaemic challenge

Kathryn Gatford[1,2], Hong Liu[1,2], Zuleeza Ahmad[1,2], Andrea Fernandez-Indulski[1,2], Miles De Blasio[1,2], Gary Heinemann[1,2], Julie Owens[1,2]
[1]Robinson Institute and [2]School of Paediatrics & Reproductive Health, University of Adelaide, SA 5000, Australia.
kathy.gatford@adelaide.edu.au

Background: Restricted growth before birth (IUGR) increases the risk of diabetes in adulthood (1), implying that insulin secretion fails to normally adapt and upregulate adequately in response to insulin resistance. Consistent with this, in adult IUGR men (2) and in placentally restricted IUGR sheep (PR) (3), insulin secretion is inadequate for the level of insulin resistance. We have developed a direct test of the plasticity of insulin secretion, using chronic mild hyperglycaemia to increase the demand for insulin in sheep (4).
Aims/Hypothesis: We hypothesised that PR would impair maintenance of glucose tolerance when challenged by hyperglycaemia in the adult sheep.
Methods: Glucose tolerance was measured before and during a hyperglycaemic (HG) or saline euglycaemic (EUG) challenge in young adult control (CON) and PR sheep at 1 year of age (CON EUG n=13, CON HG n=17, PR EUG n=10, PR HG n=10). HG sheep were infused continuously with 25% glucose at a rate adjusted daily to achieve and maintain a 50% increase in blood glucose. Outcomes were analysed in CON and PR progeny and for sheep in the lower (LBW) and upper half (HBW) of birth weight.
Results: LBW sheep exhibited higher peak plasma glucose concentrations (P=0.030) and had poorer glucose tolerance pre-challenge (P=0.019) than did HBW sheep. Glucose tolerance following challenge differed between CON and PR sheep (treatment*challenge interaction P=0.044). With a HG challenge, glucose tolerance (as a % of pre-challenge values) increased more in PR sheep than in CON sheep (CON: ↑66%, PR: ↑191%, P=0.043), but not with EUG.
Conclusions: PR sheep were less able than CON sheep to maintain glucose tolerance when challenged by chronic mild hyperglycaemia, consistent with impaired capacity to adapt glucose regulatory mechanisms. Insulin secretion measures are underway to determine whether this reflects impaired plasticity of insulin secretion.

1. **Whincup PH *et al.*** Birth Weight and Risk of Type 2 Diabetes: A Systematic Review. *JAMA* 300: 2886-97, 2008.
2. **Jensen CB *et al.*** Early differential defects of insulin secretion and action in 19-year-old Caucasian men who had low birth weight. *Diabetes* 51: 1271-80, 2002.
3. **Gatford KL *et al.*** Impaired β-cell function and inadequate compensatory increases in β-cell mass following intrauterine growth restriction in sheep. *Endocrinology* 149: 5118-27, 2008.
4. **Gatford KL *et al.*** Testing the plasticity of insulin secretion and β-cell function in vivo: responses to chronic hyperglycaemia in the sheep. *Experimental Physiology* 97: 663-75, 2012.

Angiotensin II, Angiotensin 1-7 and skin blood flow during hypoxia in the preterm piglet

Yvonne Eiby[1], Eugenie Lumbers[1,2], Michael Staunton[1], Layne Wright[1], Paul Colditz[1] & Barbara Lingwood[1].

[1]Perinatal Research Centre, UQ Centre for Clinical Research, The University of Queensland, QLD 4029, Australia, [2]Mothers and Babies Research Centre, Hunter Medical Research Institute, NSW 2305, Australia.
y.eiby@uq.edu.au

Background: Immature control of the cardiovascular system may be a contributing factor to poor cardiovascular function and associated increases in mortality and morbidity in preterm infants.

Aims/Hypothesis: This study aimed to assess the association between angiotensin II (Ang II) and angiotensin 1-7 (Ang 1-7) concentrations and skin blood flow during a mild hypoxic stress in preterm and term piglets.

Methods: Piglets were delivered by C-section at 97 and 113 days of gestation (term≈115d). An additional preterm group was exposed to maternal glucocorticoid treatment. Changes in mean arterial blood pressure (MAP), skin blood flow and plasma concentrations of Ang II and Ang 1-7 were measured in response to acute hypoxia (4% O_2 for 20min).

Results: Preterm piglets were less likely to exhibit a mature cardiovascular compensatory response to hypoxia (increased MAP and reduced skin blood flow) than term piglets. Plasma Ang II levels were reduced in glucocorticoid exposed preterm piglets compared to untreated preterm piglets (P<0.05) possibly due to increased conversion from Ang II. Glucocorticoid exposed preterm piglets also had increased skin blood flow during hypoxia. In term piglets there was no change in plasma concentrations of Ang 1-7 in response to hypoxia but glucocorticoid exposed preterm piglets with increased Ang 1-7 during hypoxia also had increased skin blood flow while those with no change or reduced Ang 1-7 had decreased skin blood flow.

Conclusions: Ang II and Ang 1-7 appear to be active in vasomotor control in the preterm piglet but levels are altered by maternal glucocorticoid treatment. Low levels of Ang II and increases in Ang 1-7 in glucocorticoid exposed preterm piglets may contribute to increases skin blood flow during hypoxia.

Intrauterine inflammation alters cardiopulmonary but not cerebral haemodynamics during open endotracheal tube suction in preterm lambs

Robert Galinsky[1], Timothy J.M. Moss[1,2], Graeme R. Polglase[1,2], Stuart B. Hooper[1,2]

[1]The Ritchie Centre, Monash Institute of Medical Research, Monash University, Victoria 3168, Australia.
[2]The Department of Obstetrics and Gynaecology, Monash University

r.galinsky@auckland.ac.nz

Background: Intrauterine inflammation adversely affects cardiopulmonary, systemic and cerebral hemodynamics in preterm neonates but the impact of stimuli known to alter pulmonary hemodynamics, such as endotracheal tube (ETT) suction, is unknown. We hypothesized that intrauterine inflammation would impair the cardiopulmonary and cerebral hemodynamic response to open ETT suction in preterm lambs.

Methods: At ~112 days of gestation (d: term is ~147 d) aseptic fetal surgery was performed for implantation of arterial and venous catheters and Transonic flow probes for real-time measurement of pulmonary, systemic and cerebral haemodynamics. At ~118 d, inflammation was induced by intra-amniotic administration of *E coli* lipopolysaccharide (LPS; 20 mg). Fetuses exposed to LPS (n=6) or saline (n=4) were delivered via Caesarean section at ~125 d. Lambs were fitted with a pulse oximeter for pre-ductal measurement of arterial oxyhaemoglobin saturation and mechanically ventilated using a positive end-expiratory pressure of 4 cmH_2O and a set tidal volume of 7.5 mL/kg. Thirty minutes after the initiation of ventilation open ETT suction was performed for 30 seconds. Pulmonary, systemic and cerebral arterial pressures and flows were continuously recorded before, during and after ETT suction. Data were compared using a two-way ANOVA with repeated measures.

Results: Pre-ductal oxygenation decreased during ETT suction ($p<0.001$), but to a greater extent in LPS-exposed lambs ($p=0.02$ v. saline). Pulmonary blood flow and left ventricular output increased during and immediately after ETT suction in both groups ($p=0.01$ and 0.001, respectively), but values were higher in LPS-exposed lambs ($p<0.01$ and 0.02 v. saline, respectively). Cerebral blood flow and systemic arterial pressure were increased by open ETT suction similarly in both groups ($p<0.001$ and 0.01, respectively).

Conclusion: Intrauterine inflammation exacerbates cardiopulmonary but not cerebral hemodynamic changes during open ETT suction. This is likely due to an inflammation-induced impairment in pulmonary vascular development and function.

Maternal creatine supplementation protects the neonatal spiny mouse following birth asphyxia, but what are the effects on the mother?

Stacey J. Ellery[1], Domenic L. La Rosa[1], Michelle M. Kett[2], Rod Snow[3] David W. Walker[1] and Hayley Dickinson[1]

[1]The Ritchie Centre, Monash Institute for Medical Research, Melbourne, Australia. [2]Department of Physiology, Monash University, Clayton, Australia 3088. [3]Centre for Physical Activity and Nutrition Research, School of Exercise and Nutrition Sciences, Deakin University, Burwood, Australia.

stacey.ellery@monash.edu

Background: Maternal dietary creatine (Cr) supplementation and fetal tissue loading has proven beneficial in ameliorating the multi-organ damage associated with birth asphyxia (Ellery et al 2012, *Pediatr Res*; Ireland et al 2011, *Neuroscience*; Cannata et al 2010, *Pediatr Res*). However, the effect of supplementation on the mother is unknown.

Aim/Hypothesis: To determine if a Cr supplemented diet effects maternal weight gain, eating/drinking behaviours or urinary electrolyte and osmotic balance during the second half of pregnancy, along with *de novo* synthesis of Cr at term.

Methods: Pregnant spiny mice were maintained on normal chow or chow supplemented with 5%w/w Cr from day 20 gestation (20dGA). Weights were recorded til term at 3-day intervals. On 23d and 35dGA dams underwent physiological assessment of food/water intake, urinary output, creatine content, osmolality and electrolyte levels. On 38dGA (term ~39dGA) kidneys, brain and liver were collected and fixed for histological analysis, or frozen for molecular analysis of the creatine transporter (CrT) and creatine synthesising enzymes arginine:glycine amidinotransferase (AGAT) and guanidinoaceteate methyltransferase (GAMT).

Results: Weight gain throughout the second half of pregnancy was not different for Cr and control diet dams (P=0.1). In addition, no alteration in food/water intake or urine output was noted with a Cr diet (P_{Food}=0.9, P_{Water}=0.8, P_{Urine}=0.9). Analysis of urinary Cr content indicated Cr supplementation increased the rate of Cr excretion, however this rate decreased in the later part of pregnancy (P_{Diet}<0.03, P_{GA}<0.001). Negative feedback regulation of *de novo* creatine synthesis, via renal AGAT, was shown with decreased AGAT protein expression in the kidney (P<0.003).

Conclusion: This study provides evidence that the maternal Cr supplementation employed in our spiny mouse model of birth asphyxia is safe for the mother, and therefore can be considered further as a potential treatment to prevent the adverse outcomes of birth asphyxia.

Extra-uterine renal growth in preterm infants: Oligonephropathy and prematurity

Yogavijayan Kandasamy [1,2], Roger Smith[2], Ian M R Wright[2], Eugenie R Lumbers[2,3]
[1]Department of Neonatology, The Townsville Hospital, Queensland 4814, Australia
[2]Mother and Babies Research Centre, Hunter Medical Research Institute, John Hunter Hospital, Locked Bag 1, Hunter Region Mail Centre, Newcastle, NSW 2310, Australia
[3]School of Biomedical Sciences, University of Newcastle, University Drive, Callaghan, Newcastle, NSW 2310, Australia
E-mail: yogavijayan.kandasamy@uon.edu.au

Background: Nephron number in humans is determined during fetal life.
Aim/Hypothesis: The objective of this study was to investigate the effects of preterm birth on postnatal renal growth using renal volume as an estimate of nephron number. We postulated that premature infants at term corrected age (CA) would have fewer glomeruli and, as a result, smaller kidney volumes than term infants.
Methods: This cross-sectional study was conducted over 12 months in a tertiary perinatal centre. Preterm babies admitted to the neonatal unit at less than 32 weeks of gestation were recruited and followed until discharge. Term infants (≥ 37 completed weeks of gestation) were recruited as controls. The babies underwent the first assessment (renal sonography and renal function measurement) at 32 weeks corrected age (CA) and a second assessment at 38 weeks CA. The primary outcome measure was total kidney volume (TKV) and the secondary outcome was glomerular filtration rate (GFR).
Results: Forty-four preterm infants and 24 term infants were recruited. At 38 weeks CA, premature infants had lower TKV than controls (21.6±5.7 vs. 25.2 ±5.7 cm^3; P = 0.02). Premature infants also had smaller body weights compared to controls (2566±406 vs. 3416±434 g; P < 0.001). However, when corrected for their smaller body weights, preterm infants at 38 weeks CA had higher kidney volumes per kg body weight ratio than controls (8.5±2.2 vs. 7.4±1.7 cm^3/kg; P = 0.03) but a significantly lower GFR (73.6 [IQR 68.1-77.6] vs.79.3 [IQR 72.5-86.6] $mL \cdot min^{-1} \cdot 1.73m^{-2}$; P = 0.03)
Conclusions: Premature infants have larger kidney volumes relative to their body weight but a lower GFR. This suggests that compensatory glomerulomegaly and tubular hypertrophy occur in preterm infants during the first postnatal weeks of development. The potential for this to lead to impaired renal function and hypertension later in life needs further investigation.

Novel urinary biomarkers for predicting pregnancy outcome in indigenous and non-indigenous Australian women

Pringle KG[1,2], Sykes SD[1,2], Weatherall L[2,3], Clausen D[4], Smith R[2], Rae K[2,3] and Lumbers ER[1,2]

[1]School of Biomedical Sciences and Pharmacy, University of Newcastle, Newcastle, NSW, Australia; [2]Mothers and Babies Research Centre, HMRI, Newcastle, NSW Australia; [3]Gomeroi gaaynggal Centre, Tamworth, NSW, Australia; [4]Pathology New England, Tamworth, NSW, Australia.

Kirsty.Pringle@newcastle.edu.au

Background: Indigenous Australian women are 2.5 times more likely to get preeclampsia in pregnancy, possibly because of a high incidence of undetected renal disease. We postulate that the intrarenal renin angiotensin system (iRAS) is activated in normal pregnancy and that failure to activate the iRAS, due to underlying renal impairment, may predispose to preeclampsia.

Aims/Hypothesis: To measure the activity of the iRAS in non-pregnant and pregnant non-Indigenous and Indigenous women.

Methods: Urinary angiotensinogen/creatinine (uAGT/creat), albumin/creat, and protein/creat were measured in 10 non-pregnant and 17 pregnant non-Indigenous women and in 61 Indigenous pregnant women recruited in Tamworth and Newcastle as part of the Gomeroi gaaynggal ArtsHealth program. In Indigenous pregnant women, uACE/creat, uprorenin/creat and uactive renin/creat were also measured. Data are presented as mean ± SEM.

Results: uAGT/creat was higher in pregnant (18.2 ± 3.2 ug/mmol, n=9) compared to non-pregnant women (1.1 ± 0.3 ug/mmol, n=10, P=0.001) but women with clinical proteinuria and/or preeclampsia had low uAGT/creat (n=3). Hypertensive women had normal high uAGT/creats (n=4).

Indigenous pregnant women had higher protein/creat (P=0.01) and lower uAGT/creat (2.9 ± 1.0 ug/mmol, n=51, P=0.01) than non-Indigenous pregnant women. Two groups of Indigenous women were identified, those with a uAGT/creat <2ug/mmol (n=37) and those with a value >2.0ug/mmol (n=12). Only the low uAGT/creat Indigenous pregnant women showed significant correlations between uAGT/creat and albumin/creat (rho 0.367, P=0.027), uactive renin/creat and both urinary albumin/creat (rho=0.493, P=0.002) and urinary protein/creat (rho=0.603, P<0.001, n=36). uAGT/creat fell with gestation (rho= -0.329, P=0.047) as did GFR (increased Cystatin C, rho=0.592, P<0.001, n=36), which was correlated with uprotein/creat (rho =0.357, P=0.03, n=36). Only in women with a normal high uAGT/creat was uNa/K inversely related to gestation age (rho=-0.456, P=0.05, n=18).

Conclusions: The iRAS is activated in pregnancy. A number of Indigenous women however, do not activate their iRAS when pregnant. As Indigenous pregnant women had higher urinary protein/creatinines, and non-Indigenous women with proteinuria or preeclampsia also had low uAGT/creatinines, a low uAGT/creat in pregnancy may be a new sensitive indicator of impaired renal function and may be associated with an increased risk of preeclampsia.

Effect of 'Cafeteria diets' on the fatty acid composition of her milk and offspring

Authors: Mini Vithayathil[1], ZY Ong[1], R Gibson[1] and Beverly Muhlhausler[1,2]
[1]FOODplus Research Centre, Agriculture, Food & Wine, University of Adelaide, Australia. [2]Sansom Institute, School of Pharmacy and Medical Sciences, Uni SA, Australia.

mini.vithayathil@adelaide.edu.au

Background: We have previously shown that cross fostering offspring onto a dam consuming a cafeteria 'junk food' diet results in increased fat mass in the offspring at weaning, independent of the nutritional environment experienced before birth. This suggested that the maternal diet during the lactation period is the most important determinant of early accumulation of body fat mass, and prompted us to determine whether this could be explained by differences in the total fat content and/or fatty acid composition of the maternal milk.
Aim: This study aimed to determine the impact of consuming a cafeteria 'junk food' diet before birth and/or during the lactation period on the fatty acid composition of the maternal milk and the fatty acid status of the offspring.
Methods: 26 dams were fed either standard rat chow (Control, n=14) or cafeteria 'high fat' diet (HF, n=12) during pregnancy and lactation. After birth, all pups were cross-fostered onto another dam from either the same or different feeding group. Milk samples were collected at 2nd week of lactation. Body weight and body fat mass in the offspring were determined at 3 weeks. Blood samples from the offspring were collected on postnatal day 1(P1) and at 3 weeks of age. Fatty acid composition of blood and milk samples were determined by gas chromatography.
Results: Compared to mothers on a control diet, mothers on the 'cafeteria' diet had a higher total fat during pregnancy and lactation. Specifically, they had higher intake of saturated fat and omega-6 polyunsaturated fats (n-6 PUFA) and omega-3 polyunsaturated fatty acids (n-3 PUFA). These differences were reflected in the maternal milk composition, which contained a higher proportion of saturated fatty acids ($P<0.01$) and n-6 PUFA ($P<0.05$) in the HF group. There were no differences, however, in the saturated fatty acid status of the pups on either P1 or 3 weeks of age, and at 3 weeks of age, pups suckled by HF dams had lower levels of both n-6 ($P<0.05$) and n-3 PUFA ($P<0.01$) compared to those suckled by Controls. There were no direct relationships between the fatty acid content of the maternal diet or the maternal milk and the fatty acid status of the offspring at either P1 or 3 weeks of age.
Conclusions: Our findings suggest that the fatty acid composition of maternal diet during gestation and lactation is directly related to the fatty acid composition of maternal milk. However, there appears to be a more complex relationship between the fatty acid intake of the pups and their fatty acid status in early life which requires further investigation.

Determining the role of glucocorticoids in inflammation-induced fetal lung maturation using glucocorticoid receptor knockout mice.

Melinda J Dolan[1], Megan J Wallace[1,2], Annie RA McDougall[1,3], Valerie A Zahra [1], Timothy J Cole [3] & Timothy JM Moss[1,2]

[1]The Ritchie Centre, Monash Institute of Medical Research, [2]The Department of Obstetrics and Gynaecology & [3]Biochemistry and Molecular Biology, Monash University, VIC 3800, Australia

melinda.dolan@monash.edu

Background: Intra-amniotic inflammation, which is common in preterm births, induces precocious fetal lung maturation. Similarly, glucocorticoids play a vital role in fetal lung maturation. However it is not known if glucocorticoids mediate the effect of inflammation on lung maturation.

Aims/Hypothesis: We used glucocorticoid receptor (GR) transgenic mice to investigate the role of glucocorticoid signalling in inflammation-induced fetal lung maturation. We hypothesised that the effects of inflammation on lung development are independent of glucocorticoids, despite their fundamental role lung maturation.

Methods: GR+/- mice were time mated. Pregnant females underwent aseptic surgery and 5μl of lipopolysaccharide (LPS; 20 pg/μl; n=4 dams) or saline (n=4 dams) were administered by intra-amniotic injection on embryonic day (E) 15.5. Fetal tissues were collected on E17.5 and fixed in 4% paraformaldehyde for histological analysis. Lung sections, cut at 5μm, were stained with haematoxylin and eosin. Analysis of lung tissue fraction is currently being performed.

Results: Representative images of E17.5 lung tissue are presented in Figure 1. Preliminary analysis suggests there is) little effect of LPS (B & D vs A & C), however as expected, lungs of GR-/- mice (C & D) have a greater tissue density than GR+/+ (A & B).

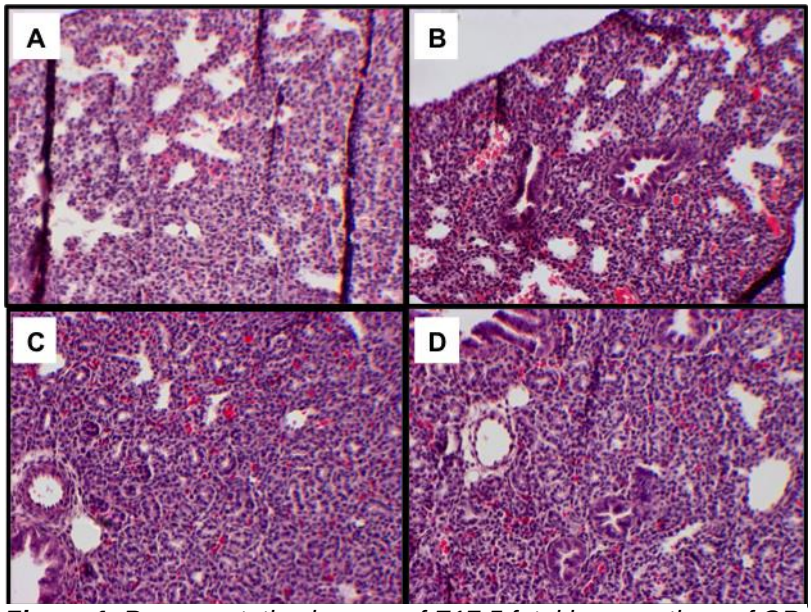

Figure 1. *Representative images of E17.5 fetal lung sections of GR +/+ (A and B) and GR -/- (C and D) after intra-amniotic injections of saline (A and C) or LPS (B and D).*

Conclusions:

There appears to be little effect of LPS on tissue airspace ratio in GR-/- fetal lung sections. However, unexpectedly, there also appeared to be very little effect of LPS on GR+/+ lungs. The reason for this unexpected result is not clear but is being investigated.

Neonatal exendin-4 normalises glucose tolerance and insulin secretion in IUGR sheep

Hong Liu[1,2], Damien Hunter[1,2,3], Gary Heinemann[1,2], Anita Peura[1,2], Himawan Harryanto[1,2], Miles De Blasio[1,2,4], Rebecca Simmons[5], Karen L Kind[1,3], Julie A Owens[1,2], Kathryn L Gatford[1,2]

[1]Robinson Institute, [2]School of Paediatrics & Reproductive Health and [3]School of Animal & Veterinary Sciences, University of Adelaide, Adelaide SA 5005, Australia; [4]Department of Physiology, Development & Neuroscience, University of Cambridge, UK; [5]Medical School, University of Pennsylvania, USA. hong.liu@adelaide.edu.au

Background: Intrauterine growth restriction (IUGR) in humans impairs adult insulin action, in part via impaired insulin secretion relative to sensitivity and hence increases the risk of diabetes[1, 2]. Similarly, IUGR due to placental restriction (PR) in sheep impairs postnatal insulin action, by reducing β-cell function and insulin sensitivity[3, 4]. PR in rats similarly induces diabetes in adult progeny, which can be prevented by neonatal exendin-4 (EX-4) treatment[5]. The latter treatment in twin IUGR lambs may also improve postnatal insulin action after IUGR in this species where the pancreas is more mature at birth[6]. However, whether insulin action in adulthood is improved by neonatal EX-4 in the PR sheep remains unknown.

Hypothesis: We hypothesise that EX-4 treatment of the neonatal IUGR sheep normalises insulin secretion, insulin sensitivity, and glucose tolerance, in adulthood.

Methods: Animals were weighed at birth and metabolic function assessed, at ~1 year of age in progeny of control ewes (CON; n = 26F, 18M), progeny of PR ewes (PR; n = 15F, 14M), and in PR progeny that were treated with EX-4 (PR+EX-4; 1 nmol/kg s.c., daily from day 1 to day 16 of age; n = 10F, 8M). Glucose tolerance and insulin secretion were assessed by intravenous glucose tolerance test (0.25 g glucose.kg^{-1}, and insulin sensitivity by hyperinsulinaemic euglycaemic clamp (120 min; 2 mU insulin kg^{-1}min^{-1}).

Results: Overall and early phase (EP) area under the glucose curve (AUC glucose) correlated negatively with birth weight in CON and PR (EP: r = -0.270, p = 0.011, n = 73), but not in PR+EX-4 (EP: r = -0.349, p = 0.101, n = 15) sheep, with similar patterns in females. Overall insulin secretion (insulin AUC, corrected for glucose AUC) in CON and PR correlated negatively with birth weight (CON and PR combined; r = -0.254, p = 0.016, n = 71), but not in PR+EX-4 (r = -0.112, p = 0.35, n = 15), with similar patterns in females where EX-4 actually reversed the association (differing slopes of regressions: p = 0.049). Insulin sensitivity also correlated with birth weight in CON and PR female progeny (r = 0.300, p = 0.045, n=33), but not in PR+EX-4 (r = -0.121, p = 0.400, n=7).

Conclusions: IUGR impairs glucose tolerance, induces hypersecretion of insulin in young adult progeny and impairs insulin sensitivity in females. Neonatal EX-4 treatment improves glucose tolerance and normalises insulin secretion in young adult IUGR sheep, particularly in females, whose insulin sensitivity is also improved.

1. Jaquet, D. *et al.* (2000) *J Clin Endocrinol Metab* 85 (4): 1401-6
2. Mericq, V. *et al.* (2005) *Diabetologia* 48 (12): 2609-14
3. Gatford, K. L. *et al.* (2008) *Endocrinology* 149 (10): 5118-27
4. De Blasio, M. J. *et al.* (2012) *Endocrinology* 153 (5): 2142-51
5. Stoffers, D. A. *et al.* (2003) *Diabetes* 52 (3): 734-40
6. Gatford, K. L. *et al.* (2013) *PLoS One* 8 (2): e56553

Widespread differential maternal and paternal genome effects on bovine fetal muscle and bone development at midgestation

Ruidong Xiang[1,2], Tanja Eindorf[2], Madeleine Gugger[2], Carolyn J. Fitzsimmons[2], Zbigniew A. Kruk[2], Mani Ghanipoor-Samami[1,2], Dana Thomsen[1,2], William H. Johns[3], Claire T. Roberts[2,4], Paul L. Greenwood[3], Brian M. Burns[5,] Gail I. Anderson[1], David L. Rutley[1] and Stefan Hiendleder[1,2]

[1]JS Davies Non-Mendelian Genetics Group, School of Animal & Veterinary Sciences, Roseworthy Campus, and [2]Robinson Institute, University of Adelaide, South Australia; [3]Department of Primary Industries, Beef Industry Centre, University of New England, Armidale, New South Wales, Australia; [4]School of Pediatrics & Reproductive Health, University of Adelaide, South Australia; [5]University of Queensland, Centre for Animal Science, Alliance for Agriculture and Food Innovation, Rockhampton, Queensland, Australia. stefan.heindleder@adelaide.edu.au

Background: Parent-of-origin effects caused by non-mendelian genetic and epigenetic mechanisms such as cytoplasmic inheritance, imprinting and miRNA interaction are emerging as important determinants of prenatal development involved in programming postnatal phenotype. We generated a bovine resource of purebred and reciprocal cross fetuses with defined *Bos taurus* and *Bos indicus* (epi)genetics to dissect maternal and paternal genome effects at the phenotypic and molecular level.
Hypothesis and aims: Maternal and paternal genomes have differential effects on phenotype of specific muscles and bones due to non-mendelian genetic and epigenetic mechanisms. Here, we aimed to identify and quantify maternal and paternal genome effects on (i) fetal myofibre characteristics, (ii) absolute and relative muscle weights and (iii) morphometric parameters and weights of bones.
Methods: Purebred and reciprocal cross fetuses (n=73) were generated with estrous cycle synchronised Angus and Brahman heifers using 3 Angus and 2 Brahman sires. Fetuses were recovered on Day153±1 post conception and *M. semitendinosus* samples frozen in liquid nitrogen for muscle fiber histology. Individual muscles and bones were dissected from vacuum packed carcasses stored at -20°C. Principal component (PC) analysis was performed for bone parameters. Parameters and extracted PCs were analysed with general linear models including effects of maternal and paternal genomes, sex and non-genetic maternal weight/daily gain effects.
Results and discussion: Maternal genetics was the predominant source of genetic variation for cross sectional area of fast myofibres (69%, $P<0.01$), while paternal genetics interacted with maternal daily weight gain ($P<0.01$) to explain genetic variation in number of fast myofibres (92%). Maternal genetics was the only source of genetic variation for absolute weight of *M. semimembranosus* ($P<0.0001$) and, in interaction with final maternal weight ($P<0.01$), explained most genetic variation in absolute weight of *M. longissimus dorsi* (62%). Paternal genetics was the only source of genetic variation in relative weight of *M. supraspinatus* ($P<0.0001$) and the predominant source of genetic variation in relative weight of *M. longissimus dorsi* (68%, $P<0.0001$). Analysis of extracted principal components revealed strong maternal genome effects on PC1/bone mass ($P<0.0001$) and PC5/axial skeletal growth ($P<0.001$), while paternal genome effects were weak (PC1/bone mass, $P<0.05$) or non-significant (PC5/axial skeletal growth, $P>0.05$). Both PC2/limb ossification ($P<0.05$) and PC4/flat bone elongation ($P<0.05$) were affected by an interaction between maternal and paternal genomes, but displayed strikingly different phenotypic expression patterns. The widespread differential maternal and paternal genome effects on fetal muscle and bone phenotype await detailed molecular studies to identify underlying (epi)genetic mechanisms.

The effect of maternal vitamin D status at 15 weeks gestation on pregnancy outcome

Sean O'Leary, Alison Leviton & Claire T. Roberts
School of Paediatrics & Reproductive Health, Robinson Institute, The University of Adelaide, SA 5005, Australia. sean.oleary@adelaide.edu.au

Background: Vitamin D is a secosteroid hormone that is best known for its role in calcium homeostasis and bone turnover. Most vitamin D is derived from the sunlight-stimulated conversion of pro-vitamin D in the skin to 25-hydroxyvitamin D3 (25D; calcidiol). Serum levels of 20nmol/L of 25D have generally been considered to be the minimum substrate level required for adequate renal synthesis of active 1,25-dihydroxyvitamin D (1,25D; calcitriol) to promote intestinal calcium absorption and prevent the bone mineralising defect of rickets. While the current recommendation for serum 25D is 60nmol/L, 80nmol/L serum 25D has been shown as a minimum level to reduce the prevalence of osteoporosis and hip fractures. **There is no information on the sufficiency levels of vitamin D in pregnancy** even though the placenta is the most prominent site of extra-renal activation of 25D during pregnancy**.** Vitamin D deficiency during early pregnancy is associated with preeclampsia (PE), intrauterine growth restriction (IUGR), gestational diabetes mellitus (GDM) and pre-term birth (PTB). Our laboratory is a partner in the **Sc**reening f**O**r **P**regnancy **E**ndpoints (SCOPE) study, which is part of the international SCOPE consortium for whom we have a wealth of social, clinical, dietary, lifestyle and pregnancy outcome data in 5,628 women. The Adelaide cohort consists of 1,169 women in which serum 25D, urinary calcium and deoxypyridinoline (DPD) as a measure of bone turnover have been measured at 15 weeks gestation in a subset of 367 women to date.

Aims/Hypothesis: To determine if maternal vitamin D deficiency and markers of calcium metabolism at 15 weeks gestation are associated with pregnancy complications including PE, PTB, IUGR, and GDM.

Methods: Maternal serum and urine samples were collected at 15 weeks gestation in 367 women attending the Lyell McEwin Hospital. 25D levels in serum and urinary DPD were measured by ELISA and urinary calcium and creatinine levels were measured by spectrophotometry using the Roche Integra 400+ System. All measurements were assessed for association with pregnancy complication.

Results: Currently we have preliminary data on a subset of 367 women with different pregnancy outcomes [PE n=50, PTB n=50, GDM n=36, gestational hypertension n=50, small for gestational age n=50, Control n=131]. We have observed differences in serum 25D due to season with lower levels in winter and with women who later developed GDM compared with uncomplicated pregnancies while those who subsequently developed PE had higher concentrations of 25D than controls (66.6±1.5 and 73.1±2.3, mean±SEM respectively). Urinary DPD:creatinine ratio was higher in women who later developed PE than for controls (PE 59.9±40.5 vs Control 9.6±0.76) although there was a lot of variability in the PE group.

Conclusions: In this preliminary study, the higher levels of DPD:creatinine ratio in women that later developed PE is suggestive of increased bone resorption to maintain serum calcium levels. In addition, lower maternal status of 25D at 15 weeks gestation was shown to be associated with GDM. Further analyses of the remaining 802 women of the Adelaide cohort of the SCOPE study will provide further statistical power to assess fully the effect of maternal vitamin D and calcium metabolism on pregnancy outcome.

The effect of carotid blood flow steal in asphyxiated near-term lambs

Kristina Sobotka[1], Graeme Polglase[1, 2], Tracey Ong[1], Tim Moss[1, 2], Stuart Hooper[1, 2].
[1]The Ritchie Centre, Monash Institute of Medical Research, Monash University, Victoria, Australia
[2]Department of Obstetrics and Gynaecology, Monash University, Victoria, Australia.
Kristina.sobotka@monash.edu

Background: In a recent study, we found that some asphyxiated near-term lambs after successful resuscitation and return of spontaneous circulation, developed retrograde diastolic carotid blood flow. Retrograde diastolic blood flow in major arteries is a sign of "ductal steal", where there is a significant shunting of blood from the descending aorta to the pulmonary circulation via an open ductus arteriosus. The resultant reduction in cerebral blood flow can cause hypoperfusion and ischemia in the brain, which has been strongly associated with intraventricular haemorrhage and white matter injury in newborns. While the presence of ductal steal in infants has been known for some time, its development and impact is still not well understood.
Aims/Hypothesis: Our aim was to compare the cardiopulmonary physiology in asphyxiated near-term infants with and without retrograde diastolic carotid blood flow.
Methods: Asphyxia was induced in instrumented fetal sheep (~140 d GA) by umbilical cord occlusion until mean arterial pressure decreased to ~22 mmHg. Lambs were then given an initial sustained inflation for 30 s at 35 cmH_2O, then mechanically ventilated for 30 min. Continuous recordings of ventilation parameters, cerebral tissue oxygen saturation, carotid arterial blood flow and pressure, pulmonary blood flow and jugular venous pressure were analysed.
Results: 6 lambs developed retrograde diastolic carotid blood flow ~15 min after the initiation of ventilation (ductal steal; n=6). These lambs were then compared to lambs that didn't develop retrograde carotid blood flow (no ductal steal; n=6). Lambs that developed ductal steal had lower fetal carotid blood flow than lambs that didn't develop ductal steal. There were no differences in arterial blood flow or pressure between the two groups at the end of asphyxia, before the initiation of ventilation. During ventilation, there were no differences in ventilation pressures (peak, mean or PEEP) or tidal volume between groups. Carotid blood flow was significantly lower from 5 min after initiation of ventilation in lambs that developed ductal steal. Carotid arterial pressure and pulmonary blood flow was similar between groups. Cerebral vascular resistance was higher in ductal steal lambs from ~ 10 min after initiation of ventilation compared to lambs without. Cerebral tissue oxygen saturation was lower in lambs with ductal steal than lambs without from 15 min of ventilation. Jugular venous pressure was higher in lambs with ductal steal compared to lambs without.
Conclusions: Lambs with low carotid blood flow during initial resuscitation following asphyxia were more likely to develop ductal steal. During ductal steal, lambs had lower carotid blood flow and reduced cerebral oxygenation, highlighting the potential dangers of this physiological state. Higher cerebral vascular resistance in ductal steal lambs likely underlies the initiation of retrograde diastolic carotid blood flow.

Impaired maternal glucose tolerance alters nephron endowment and impairs renal function in male offspring

Stacey Hokke[1], James Armitage[1,2], John Bertram[1] and Luise Cullen-McEwen[1]

[1]Dept of Anatomy & Developmental Biology, Monash University, Clayton, VIC 3800, Australia

[2]School of Medicine (Optometry), Deakin University, Waurn Ponds, VIC 3216, Australia

stacey.hokke@monash.edu.au

Background: Animal studies indicate a nephron deficit and reduced renal function in offspring exposed to a diabetic intrauterine environment. Current literature is however limited to models of persistent severe hyperglycemia and fetal growth restriction which do not reflect the typical metabolic abnormalities of human maternal diabetes.

Aims/Hypothesis: To assess the effect of impaired glucose tolerance in pregnancy on offspring nephron endowment and adult renal function.

Methods: Impaired maternal glucose tolerance was induced in C57Bl6 mice by high fat feeding (21% fat w/w) for six weeks prior to pregnancy and throughout gestation. Control mice were fed a normal matched diet (6% fat w/w). Offspring of dams with impaired glucose tolerance (IGT) or normal glucose tolerance (NGT) were collected at postnatal day (PN) 21 for determination of nephron number using unbiased stereology. Metabolic function was assessed in adult litter-mates at 6 months of age by glucose tolerance test (GTT) and body composition by dual-energy X-ray absorptiometry. Renal function was assessed by transcutaneous measurement of glomerular filtration rate (GFR) in conscious mice.

Results: Male offspring of IGT dams were found to have 31% more nephrons than offspring of NGT dams at PN21 (NGT 14,403±578 n=6 vs IGT 18,818±495 n=8; $p<0.0001$). Preliminary data shows male offspring of IGT dams to have no difference in bodyweight, body fat percentage, or glucose tolerance at 6 months of age compared to offspring of NGT dams. Renal function studies show adult offspring of IGT dams to have a 40% reduction in GFR compared to age matched control offspring of NGT dams (NGT 7.66±0.81 ul/min/g n=3 vs. IGT 4.64±0.58 ul/min/g n=3; $p=0.019$).

Conclusions: This is the first study to assess renal development in offspring exposed to impaired maternal glucose tolerance. We show that despite significantly more nephrons, these offspring show marked renal dysfunction in adulthood. Our results indicate offspring of dams with impaired glucose tolerance have perturbed renal development and subsequent programming of renal disease. Further studies are required to assess glomerular pathology including proteinuria, glomerular hypertrophy and glomerulosclerosis in adulthood.

Effects of oxygen on the expression of hypoxia inducible factors (HIFs) in first trimester placenta

Sultana Khoda[1], Amanda Highet[1], Sam Buckberry[1], Tina Bianco-Miotto[1] and Claire Roberts[1]

[1]School of Paediatrics and Reproductive Health, Robinson Institute, The University of Adelaide, Australia 5005
sultana.khoda@adelaide.edu.au

Background: Hypoxia is essential for the development of early human placenta and the fetus. During first trimester, endovascular cytotrophoblast (CTB) cells invade into and occlude maternal spiral arterioles which maintains a low O_2 environment (1%). At the end of first trimester (10-12 weeks of gestation), the maternal endothelium and smooth muscle are remodelled and the plug is displaced permitting blood flow and increased O_2 concentration (5%) to the intervillous space. Placental hypoxia in late gestation is associated with pregnancy complications such as preeclampsia and intrauterine growth restriction. Hypoxia inducible factors (HIFs) are transcription factors which mediate cellular responses to O_2. In low O_2, HIFs are translocated to the nucleus where they bind to hypoxia response elements of genes which facilitate adaptation to low O_2. In preeclamptic placentas HIF-2α is increased compared to normal term placentas.
Aim: To localise HIF-1α and HIF-2α protein in early (< 9 weeks of gestation) and late (> 9 weeks of gestation) first trimester placenta and identify HIF-regulated genes in trophoblasts.
Methods: Human first trimester placenta samples collected from elective terminations were fixed in 4% paraformaldehyde and paraffin embedded. HIF-1α and HIF-2α were assessed by immunohistochemistry on placental sections. HTR8/SVneo first trimester trophoblast cells were exposed to 1% and 5% O2 for 6 hours for gene expression analysis by microarray using Illumina HT-12 v-4. Immunocytochemistry was performed on HTR8/SVneo cells to observe the localisation of HIF-1α protein in 1% and 5% O_2.
Results: Both HIF-1α and HIF-2α protein were localised in the nucleus and cytoplasm of syncytiotrophoblast (STB), villous CTB and extravillous CTB (EVT) of early first trimester placenta. In late first trimester, the expression of HIF-1α and HIF-2α protein was observed predominantly in the cytoplasm of STB, villous CTB and EVT. HIF-1α was detected in the nucleus of HTR8/SVneo cells which were exposed to 1% O_2 but not 5% O_2. Microarray analysis identified 290 genes differentially expressed in 1% compared to 5% O_2. Ingenuity Pathway Analysis identified that 40 of these genes were HIF regulated.
Conclusion: Both HIF-1α and HIF-2α are differentially expressed in the placenta during early and late first trimester which likely reflects the O_2 environment at these times. Distribution of HIFs also indicates that HIFs are more active in early first trimester placenta compared to late. Establishing which HIF regulated genes are involved in early placenta development may identify key pathways that are altered in pregnancy complications.

Placental neurosteroidogenesis and neonatal outcome following preterm birth

Cumberland A[1,2], Hirst JJ[1,2], Kelleher MA[1,2], Palliser HK[1,2]
[1]School of Biomedical Sciences and Pharmacy, University of Newcastle, Australia,
[2]Mothers and Babies Research Centre, HMRI, Newcastle, Australia
angela.cumberland@uon.edu.au

Background: The placenta has a key role in the production of protective neurosteroids such as allopregnanolone. Chronic insults raise the expression of enzymes 5α reductase (5αR) type1 and 2, and allopregnanolone production, potentially a protective mechanism.
Aims/Hypothesis: We hypothesized the neurosteroidogenic capacity of the placenta may have contributed to the degree of neurodevelopment and neonatal outcome in preterm guineas pigs. We assessed neonatal neurosteroidogenic capacity of the placenta and neonatal brain by 5αR mRNA expression and allopregnanolone concentrations. We then compared this to neonatal outcome and assessed if sex affects these parameters.
Methods: Guinea pigs were delivered via caesarean section at term (GA69; n=22) and preterm (GA62; n=44) and maintained for 24 hours to allow transition of neuroactive steroids to neonatal levels. Preterm pups not surviving this period formed a preterm non-survivor group (n=22). Placental and brain 5αR mRNA expression determined using real-time PCR; and placental allopregnanolone concentrations were determined using radioimmunoassay. Myelination assessed by MBP immunolabelling.
Results: Preterm neonates were neurodevelopmentally immature with significantly less MBP immunolabelling in the sub-cortical and hippocampal CA1 regions than term neonates ($P<0.01$). No differences were found in placental, body and organ weight of preterm survivors and non-survivors, however respiratory function was poor. Placental 5αR2 expression was higher in preterm than term neonates ($P<0.0001$), and was also higher in non-survivors compared to surviving neonates ($P=0.04$). Non-surviving preterm male neonates also had significantly higher placental allopregnanolone concentrations than term neonates ($P=0.02$), but no differences were found between females. There were no differences in brain 5αR mRNA expression.
Conclusions: The finding that 5αR expression was greatest in preterm placenta supports the essential role of the placenta in neurosteroid production prior to term. The observation that placental 5αR2 was higher from neonates that did not survival the 24hr period suggest previous compromise that may have contributed to their poor outcome. The differing placental allopregnanolone concentrations of males but not females, is consistent with preterm non-surviving males may having a greater incidence of in utero compromise and susceptibility to adverse neonatal outcome. Further investigation is required to determine if placental neurosteroid levels are an early marker of compromise and subsequent poorer outcome.

Dietary vitamin D and calcium restriction and its effects on murine placental morphometry

Jessica Laurence[1,2], Paul Anderson[3], Claire Roberts[1,2]
[1]Placental Development Research Group, Dept of Obstetrics & Gynaecology, The University of Adelaide, SA, Australia, [2]The Robinson Institute, [3]School of Pharmacy and Medical Sciences, Division of Health Sciences, University of South Australia, SA, Adelaide
jessica.laurence@adelaide.edu.au

Background: Vitamin D (vitD) deficiency has been associated with a wide range of pregnancy complications which feature poor placental development. VitD is best known for its role in calcium (Ca) homeostasis; however it also regulates 3% of the genome through the vitamin D receptor (VDR). VDR, as well as vitamin D activating enzymes, are ubiquitously expressed in the placenta. Currently little is known about vitD's role in placental development. This study examines the effects of dietary vitD and Ca deficiency on placental morphogenesis during murine pregnancy.
Aims/Hypothesis: We hypothesise that fetal and placental weights will be reduced, with altered placental morphology in response to vitD and Ca deficiency.
Methods: This study involved feeding 3 week old C57BI6 mice one of 4 diets with normal or low vitD and Ca (1000 IU or 0 IU/kg vitD, 1% or 0.1% Ca) for 9 weeks. These mice were mated, then sacrificed at day 18.5 of pregnancy. The fetal and placental weights were recorded, with two placentas per litter fixed for histology. Sections were stained with Masson's Trichrome or immunohistochemically double labelled with anti-cytokeratin and anti-vimentin to quantify structural correlates of function. All analyses were corrected for litter size.
Results: Fetal and placental weights were unchanged between mice consuming sufficient and deficient vitD and Ca. Surprisingly, the fetal to placental weight ratios were higher for mice consuming low Ca compared to control (P=0.018, 12.263 v 11.488). Suggesting that mice on Ca deficient diets have more efficient placentas. Masson's Trichrome analysis showed that the ratio of placental labyrinth (exchange) to junctional (stem cell) area was lower in mice fed either low Ca or vitD (P=0.019; trend, P=0.054), indicating that placentas from deficient mice had proportionally less junctional zone area for feto-maternal exchange, which does not explain how they could produce heavier fetuses. Morphometric assessment of the composition of the placental labyrinth was undertaken following immunohistochemistry. Lower vitD consumption resulted in increased maternal blood space and fetal capillary densities (P<0.01), while lower Ca consumption reduced the volume density of fetal capillaries, with a thinner trophoblast layer (P<0.001), which reduces the barrier thickness of trophoblast for exchange. This suggests that the placentas from deficient diets can adapt by altering labyrinth composition and by reducing the distance for feto-maternal exchange.
Conclusions: Increased volume density of the maternal blood space may indicate increased placental perfusion which one would expect to increase fetal growth. In addition a thinner barrier to diffusion would also enhance placental transport.

Omega-3 fatty acid treatment stimulates proliferation of the placental trophoblast cell line HTR8/SVneo

Jing Zhou[1], Amanda Highet[2,3], Tina Bianco-Miotto[2,3], Claire Roberts[2,3], Stefan Hiendleder[3, 4], & Beverly Muhlhausler[1].
[1] FOODplus Research Centre, School of Agriculture, Food & Wine, University of Adelaide, SA 5064, Australia, [2]School of Pediatrics and Reproductive Health, Robinson Institute, University of Adelaide, SA 5005, Australia, [3]Robinson Institute University of Adelaide, SA 5005, [4]School of Animal & Veterinary Science, University of Adelaide, SA 5005.
j.zhou@adelaide.edu.au

Background: Previous clinical studies have demonstrated that maternal omega-3 long chain polyunsaturated fatty acid (n-3 LCPUFA), mainly Docosahexaenoic acid (DHA), supplementation is associated with a significant increase in gestational length (P=0.05). The underlying mechanisms are not well understood, and we hypothesised that this is due to effects of DHA on the differentiation and/or function of the developing placenta. Proliferation of invasive extravillous cytotrophoblasts from the tips of the placental villi is an importanant step in early placental development and contributes to a sucessful pregnancy outcome.
Aims: The aim of this study was to investigate the effects of DHA on placental development by studying the effect of DHA treatment on proliferation and gene expression in the human first trimester extravillous cytotrophoblast cell line HTR8/SVneo.
Methods: HTR8/SVneo cells were treated with either a high-DHA (50μM) lipid emulsion (DHA group) or an equivalent volume of a lipid emulsion with a fatty acid composition which reflected the typical Australian diet (control group). After 16 hours, the effect of these treatments on proliferation was determined by using a Calcein-AM assay. To measure the effect on gene expression, HTR8/SVneo cells were seeded in 6-well plates (200,000 cells/well) and cultured for 24 hours with either no treatment, 50μM DHA or control emulsion. After 24 hours, RNA was extracted from the cells and differential gene expression between groups was assessed using Illumina Human HT-12 version 4 microarrays.
Results to date: After 16 hours treatment, the amount of DHA accumulated in the cells was 24.6% in the DHA group which was significantly higher than 0.9% in the control group (P<0.001).DHA treatment increased the rate of proliferation by 145% compared to control. RNA has been extracted from cells after 24 hours treatment and we are currently awaiting microarray results.
Expected Outcomes: This study has provided evidence that DHA treatment increases the proliferation rate of a placental trophoblast cell line *in vitro.* When completed, this work will provide novel insights into the mechanisms by which maternal DHA supplementation increases gestational length and has potential applications in the design of interventions to prevent preterm birth.

Gene expression control by histone modifications in the human amnion

Tamas Zakar[1,2,3], Jonathan Hirst[1,3], Carolyn Mitchell[1,3]
[1]Hunter Medical Research Institute, NSW 2305, [2]Department of Obstetrics and Gynaecology, John Hunter Hospital, NSW 2305, [3]The University of Newcastle, NSW, 2308.
Tamas.Zakar@newcastle.edu.au

Background: Inflammatory genes are upregulated in the fetal membrane at birth. Early activation of the inflammatory genes leads to preterm birth, which is a major health problem worldwide. The mechanisms that suppress labour-associated gene expression during pregnancy and allow upregulation at term are unknown.
Aims/Hypothesis: We hypothesise that inflammatory genes in the fetal membranes are inhibited during pregnancy by repressive epigenetic histone modifications. These histone modifications change to a gene-activating pattern at term, which allows gene expression leading to labour onset. Blocking the change of histone modifications inhibits gene expression and may potentially prevent preterm birth. We have tested these hypotheses by determining the levels of the gene-activating histone-3 lysine-4 trimethylation (H3K4me3) and H3 acetylation (H3Ac) and the repressive H3 lysine 27 trimethylation (H3K27me3) at the promoters of three inflammatory genes (*PTGS2, BMP2 and IL8)* suppressed at early gestation and upregulated at term in the amnion. We have also determined the effects of IL1 stimulation and histone modifying enzyme inhibitors on *PTGS2* expression and histone modification levels at the *PTGS2* promoter.
Methods: Histone modification levels were determined by chromatin immunoprecipitation (ChIP), gene expression was measured by quantitative RT-PCR and the effects of IL1 and enzyme inhibitors were defined in amnion tissue explants.
Results: The promoters of the inflammatory genes were marked bivalently by the activating H3K4me3 and the repressive H3K27me3 modifications. Bivalency was confirmed in double-ChIP experiments. The ratio of H3K4me3:H3K27me3 levels increased markedly at all three gene promoters at term as compared to early (16-18wks) pregnancy. IL1 (10ng/ml for 24h) increased PTGS2 mRNA expression and the H3K4me3:K27me3 modification ratio at the PTGS2 promoter. The histone methyl transferase inhibitor 3-deazeneplanoicin (DZNep) and the histone acetyltransferase inhibitor anacardic acid reduced the basal and IL1-stimulated PTGS2 mRNA expression. At the same time, anacardic acid decreased and DNZep blocked the IL1-provoked increase of the H3K4me3/H3K27me3 ratio at the PTGS2 promoter.
Conclusions: Labour-associated inflammatory genes in the fetal membranes are marked bivalently by activating and suppressive epigenetic histone modifications. The modification pattern is suppressive in early pregnancy and shifts toward activation at term. Proinflammatory stimulation alters the histone modification pattern allowing the increase of PTGS2 gene expression. The pharmacological inhibition of histone modifying enzymes reduces PTGS2 expression by blocking the epigenetic activation of the gene. Histone modifying enzyme inhibitors may prevent fetal membrane activation and may be useful to decrease the risk of preterm birth.

The human placenta expresses multiple glucocorticoid receptor isoforms and expression is altered by fetal sex and maternal asthma

Zarqa Saif[1], Nicolette A Hodyl[1], Eleanor Hobbs[1], Astrud Tuck[1], Annette Osei-Kumah[1], Vicki L Clifton[1]
[1]Robinson Institute, University of Adelaide, Adelaide, SA

vicki.clifton@adelaide.edu.au

Background: We have previously identified sex-specific differences in the fetal response to cortisol. From this body of work, male foetuses appear to induce a state of glucocorticoid resistance in an environment of excess glucocorticoids while females remain sensitive to changes in glucocorticoid concentration. Our recent studies suggest that this differential response to cortisol is driven by differences in glucocorticoid receptor (GR) protein function rather than changes in GR gene transcription or GR protein expression.
Aims: In the current study we aim to define the sex-specific expression and intracellular locality of the human GR isoforms in fetal-placental tissues of normal pregnancies and pregnancies complicated by maternal asthma.
Methods: Term placentae were collected from healthy pregnant women and women with asthma. Protein was extracted for Western blot analyses.
Results: The GR antibody identified 12 specific bands in protein extracts of whole placenta at a molecular weight (MW) of 94, 91, 81, 74, 69, 68, 65, 60, 55, 50, 45 and 38 kDa. Some of these MWs are equivalent to known isoforms including GRα (94kDa), GRβ (91 kDA), GRα C (81 kDa) GRP (74kDa) GRA (65 kDa), GRα D1-3 (45-55 kDa). Some isoforms have not been previously reported including the 69, 68, 60 and 38 kDa proteins. In the presence of asthma there was increased expression of cytoplasmic GRα D (45 kDa) in male and female placentae. Nuclear expression of GRα D (45 kDa) was significantly increased in female placentae in the presence of asthma. In male placentae of pregnancies complicated by asthma, cytoplasmic GRβ was significantly increased.
Conclusion: Glucocorticoid sensitivity in placentae of female foetuses may be mediated by GRα D isoform and glucocorticoid resistance in male pregnancies may be mediated by the dominant negative effects of GRβ.

Authors are listed as surname, initial/s……..abstract number/s