34th Annual Meeting of the FNWANZ Abstract Booklet

Was to be at:
Rydges Newcastle
Newcastle, New South Wales
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Now POSTPONED to:
Rydges Newcastle
Newcastle, New South Wales
8th-9th February 2021

2020-2021 Organising Committee

Principal Organising Committee
Dr Robert De Matteo

Local Organising Committee
Dr Julia Shaw
Dr Hannah Palliser
Prof Jonathan Hirst
A/Prof Kirsty Pringle

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Scientific Program

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

Terms of reference

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

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

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Involvement of Bromodomain and Extra-terminal (BET) Epigenetic Reader proteins in the regulation of inflammatory genes in decidual stromal cells

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Background: Accumulated evidence suggests that immune cell trafficking and activity in the decidua is controlled by decidual stromal cells (DSCs). DSCs function to maintain pregnancy throughout most of gestation, but at term they adopt an inflammatory phenotype that facilitates parturition. The mechanism that underlies this switch may involve epigenetic changes that regulate inflammatory gene expression.

Hypothesis: We hypothesised that inflammatory genes in DSCs are epigenetically regulated by BET family of epigenetic reader proteins, which specifically recognize lysine-acetylated histones at gene regulatory regions of the chromatin *via* bromodomains.

Methods: Decidua tissue was isolated from term placentae after elective caesarean section. Cells were dispersed by enzyme digestion, and DSCs were purified by PERCOLL gradient separation and immune-magnetic selection. Purity was confirmed by flow-cytometry. Sub-confluent primary DSC cultures were treated with the BET-BRD inhibitor compounds (+)-JQ1, I-BET762 and the inactive control compound (-)-JQ1 at 0.5-1 mM for 48 hrs as recommended by Structural Genomics Consortium (SGC). The cells were stimulated subsequently by *Lipopolysaccharide (LPS, 1*mg/ml*)* for 24hrs and the expression of prostaglandin synthetic enzymes PTGS1, PTGS2, PGES; inflammatory factors IL6, IL8, TNF-a and anti-inflammatory factors IL10 and IDO1 was determined by qRT-PCR. Expression of BET isoforms BRD2, -3 and -4 in DSCs was established by qRT-PCR. Acetyl histone-3 and acetyl histone-4 levels and the binding of BRDs at gene promoters were determined by chromatin immunoprecipitation (ChIP). Results were analysed by repeated measures ANOVA and Bonferroni adjustment of P-values (<0.05 for significance, adjusted).

Results: LPS stimulated the expression of *TNF-a, IL6, IL8, IL10, IDO1* and *PTGS2* ($p<0.05$) but not of *PTGS1* and *PGES*. The BET-BRD inhibitors reduced the basal and LPS-induced expression of *PTGS2, IL6, IL10* and *IDO1* ($p<0.05$). In contrast, basal and LPS-induced expression of *IL8* and *TNF-a* was unaffected by BET-BRD inhibitors. *BRD2* and *BRD4* were highly expressed in DSCs, while *BRD3* mRNA abundance was low. Preliminary ChIP data (N=2-4) suggested that LPS treatment robustly increased histone-3 and -4 acetylation and BRD4 and BRD2 binding at the *IDO1, IL6* and *IL10* promoters.

Conclusions: Bacterial endotoxin (LPS) stimulates the expression of both pro- and anti-inflammatory genes in DSCs. A subset of these genes is regulated by epigenetic mechanisms involving LPS-induced histone acetylation and BRD binding to the promoters. The results suggest that DSCs respond to infectious insults in a balanced fashion to avoid an extreme inflammatory reaction that may induce untimely birth.

Effect of branched chain amino acids (BCAA) supplements on pancreatic function of preterm-born lambs at one year of age

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Background: Preterm birth is associated with impaired pancreatic development. BCAA are key nutrients for the development of the endocrine pancreas, especially insulin secreting β-cells.

Hypothesis: We hypothesised that neonatal BCAA supplementation in preterm born lambs would enhance β-cell development and function.

Methods: Preterm lambs (137 days of gestation) were randomised to receive daily oral supplements containing BCAAs (leucine, isoleucine and valine in a ratio of 1: 1.1: 1.9), calorie-equivalent maltodextrin, or volume-equivalent water for two weeks after birth. Term Control lambs received volume-equivalent water. At 12 months of age, a hyperglycaemic clamp with arginine challenge was performed to assess insulin sensitivity and secretion. Groups were compared using ANOVA and Tukey HSD testing was applied. Data are mean±SEM.

Results: There was no difference amongst groups in baseline glucose or insulin concentrations, insulin sensitivity or response to arginine challenge. In both preterm supplemented groups, but not in term or preterm water controls, males had greater first phase insulin release than females (Table 1). Response to arginine followed a similar pattern.

	Preterm (*n* = 42)									Term (*n* = 12)		
	BCAA (*n* = 7♂ / 7♀)			Maltodextrin (*n* = 7♂ / 8♀)			Water (*n* = 6♂ / 7♀)			Water (*n* = 5♂ / 7♀)		
	Male	Female	*P*	Male	Female	*p*	Male	Female	*p*	Male	Female	*p*
FIR-AUC (µg/L/min)	48±7	27±4	**0.01**	43±9	21±3	**0.03**	34±7	27±5	0.3	33±8	27±6	0.5
SIR-AUC (µg/L/min)	213±23	179±28	0.3	263±53	161±24	0.09	203±43	178±52	0.7	199±68	158±32	0.9
AC-AUC (µg/L/min)	174±16	148±15	0.2	199±34	134±19	0.1	169±40	160±46	0.8	136±31	130±21	0.8

Table 1. FIR-AUC, first phase insulin response-area under curve; AC-AUC, arginine challenge-area under curve; SIR-AUC, steady state insulin response-are under curve

Conclusions: Brief nutritional supplementation of preterm born lambs with either BCAA or maltodextrin had specific effects on β-cell function into young adulthood in males only. Targeted nutritional manipulations to mitigate effects of preterm birth on organ development may have unforeseen sex-specific metabolic outcomes.

Ibuprofen does not restore fetal breathing movements following inhibition by intrauterine inflammation

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Background: Preterm infants exposed to infection and inflammation in the womb have an increased need for respiratory support at birth. The brainstem is the critical control centre for breathing and is highly vulnerable to inflammation. Research shows that high prostaglandin E2 (PGE_2) levels (inflammatory mediator) is associated with apnoea.

Aims: We aimed to determine whether antenatal blocking of PGE_2 using Ibuprofen improves fetal breathing movements (FBMs) following intrauterine inflammation.

Methods: Fetal lambs at 125±1 days gestation were instrumented to measure fetal respiratory function, heart rate, blood pressure, and blood gases. At 129±1 days gestation, fetal lambs received saline or escalating doses of Lipopolysaccharide (LPS; 300ng, 600ng, 1.2ug; I.V) over 3 days, or LPS + ibuprofen (5mg/kg/d; I.V; 1 hr following LPS exposure). FBMs were assessed using LabChart. PGE_2 levels in the brainstem, cerebrospinal fluid (CSF) and plasma were assessed by ELISA.

Results: LPS exposure caused a significant reduction in FBMs compared to saline control, and an increase in PGE_2 levels within the CSF, and transient increases within plasma. LPS exposure caused a reduction in SaO_2, PaO_2, and pH, and increased $PaCO_2$ and lactate levels. Ibuprofen did not restore FBMs, despite the reduction in PGE_2 levels, and did not improve fetal blood gases.

Conclusions: LPS exposure decreases FBMs, increases PGE_2 levels within the CSF, and to a lesser extent blood plasma, and alters fetal blood gases. Our data show that Ibuprofen does not restore FBMs and suggests that other mechanisms are involved.

Effect of high fat diet (HFD) induced paternal obesity and micronutrient intervention on metabolic outcome of grand offspring

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Background: Emerging evidence in rodents including work from our lab suggests that high fat diet (HFD) induced paternal obesity can predispose subsequent generations to metabolic and reproductive complications.

Aims/Hypothesis: Here, we aimed to investigate transgenerational effects of paternal obesity on the metabolic health of grand offspring. We designed a novel micronutrient supplement (consisting of metabolites of one carbon metabolism) to ameliorate any transgenerational effects.

Methods: Founder (F0) male Sprague Dawley rats (12 per group, 48 total) were weaned (Day21) onto control (C) or HFD (H), or micronutrient supplemented versions of these (CS; HS). At 19 weeks of age they were mated with chow fed females. 48 F1 male offspring (one from each F0 pairing) were weaned onto C, generating four F1 groups. They were mated with chow fed females at 17 weeks of age. Male and female F2 were fed C or H from weaning and underwent Echo MRI and oral Glucose Tolerance Test (oGTT) after 6 and 8 weeks on diet, respectively.

Results: In founders, HFD significantly increased body weight (H: 765±16 vs C: 632±15 g; p= 0.0001) which was normalized by supplementation (HS: 601±11 g vs H; p=0.0001). Feeding H or HS to F0 males had no effect on the adiposity and glucose homeostasis of male F1 offspring (fathers of F2). In F2 males, post weaning HFD increased adiposity and glucose intolerance. Male grand offspring from supplemented grandfathers had reduced fat mass if grand offspring themselves were challenged by HFD. However, this finding was not evident when considering fat as percent body weight. Overall significant effect of F0 HFD was observed on F2 male fat as percent body weight and net fat mass indicating F2 males from F0 (H or HS fed) had reduced fat mass and fat percentage. oGTT in F2 males revealed an overall significant effect of F0 supplementation suggesting decreased glucose tolerance in F2 males. No effect of F0 HFD was observed on glucose homeostasis of male grand offspring. In F2 females, post weaning HFD increased adiposity and glucose intolerance. No effects of F0 H or HS diet were observed on body weight or adiposity of F2 females. However, HFD fed F2 females sired by HFD-F0 had reduced glucose tolerance at 15min during oGTT.

Supplementation of the grand paternal HFD reduced glucose tolerance in F2 females (on CD or HFD).

Conclusions: Our results suggest grandfathers' HFD can impair glucose homeostasis of female grand offspring, if female grand offspring are themselves challenged by HFD and may reduce adiposity of male grand offspring indicating a sex specific transgenerational effect. Dietary micronutrient supplementation reduced adiposity in fathers but could impair glucose homeostasis of male or female grand offspring.

Measurement of the fetal renal parenchyma to assess the effects of fetal growth restriction on the developing kidney

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Background: Fetal growth restriction can adversely impact kidney development resulting in a reduced nephron number and increased risks for hypertension and chronic kidney disease. To date, no study has explored the development of the fetal renal parenchyma as a surrogate for nephron numbers.

Aims: The aim of this study was to compare the renal parenchymal thickness between fetal growth restriction (FGR) and appropriately grown (AGA) fetuses during pregnancy.

Methods: A longitudinal, observational study was conducted between May 2017 to February 2019. Mixed-risk women with a singleton pregnancy underwent an ultrasound scan at least every four weeks between 16 and 38-weeks. Renal parenchymal thickness, renal artery Doppler and fetal biometry were assessed in AGA and FGR fetuses and were analysed using mixed-effects modelling.

Results: We recruited 102 AGA and 30 FGR fetuses. The renal parenchymal thickness was found to be significantly thinner in FGR compared to AGA fetuses (LR=21.06, p=<0.0001). FGR fetuses were found to have a thinner parenchyma than an AGA fetus, regardless of head circumference (LR=8.9, p=0.0028) and had a slower growth trajectory. This supports the principle that FGR fetuses preferentially shunt blood towards the brain. The renal artery blood flow between the two groups showed no significant difference.

Conclusions: Our study demonstrates that FGR negatively influences nephron numbers as it is associated with a thinner parenchyma and slower growth trajectory. Measurement of the renal parenchymal thickness has the potential to identify newborns with a possible reduced nephron endowment, facilitating monitoring and early intervention to reduce future kidney disease.

Prenatal stress programmes persisting GABAergic and glutamatergic disruption in the developing hippocampus

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Background: The neuron-specific K-Cl co-transporter, KCC2, maintains low expression in early development and its upregulation is associated with the maturation of GABAergic hyperpolarising action. Due to its important role in GABAergic inhibition, disruption to this system has been implicated in neurological disorders.

Aims: To examine KCC2 expression, and markers of the GABAergic and glutamatergic systems in the guinea pig hippocampus in the second half of gestation and to determine how prenatal stress disrupts these pathways.

Methods: Pregnant guinea pigs were subjected to psychosocial stress (strobe light exposure, 2 hours a day every 5 days, from gestational days (GA) 35-65), with fetal tissue collected on GA 40, 50, 60 and 69 (term GA71). A cohort of control and prenatally-stressed offspring were allowed to deliver and tissue collection was conducted at 1 month of age. Real-time polymerase chain reaction (qPCR) was conducted with frozen hippocampal tissue to assess relative mRNA expression of key components of the GABAergic and glutamatergic pathways.
Results: K-Cl cotransporter, KCC2 (SLC12A5) mRNA, underwent a significant developmental upregulation in the hippocampus between GA40 and GA50 in control guinea pigs, which remained relatively stable for the remainder of gestation, however, stress resulted in a significant decrease in SLC12A5 mRNA expression at GA60. GABRA1:2, GABRA3, GABRA4 and SLC1A3 mRNA expression were also significantly downregulated at GA60 following repeated prenatal stress exposure. Examination of these prenatally-stressed offspring at 1 month of age also revealed large disruptions to components of the GABAergic and glutamatergic systems.

Conclusions: In the guinea pig, KCC2 appears to undergo a developmental upregulation during mid-late gestation (GA40-50), indicating a prenatal shift of GABAergic transmission from depolarising to hyperpolarising, a similar trajectory to human GABAergic development. Disruption of KCC2 upregulation at GA60 following prenatal stress suggests reduced GABAergic inhibitory action. Also that stress-induced impacts on GABAergic and glutamatergic components in fetal life programmes for disruption to the excitatory/inhibitory balance into postnatal life, and subsequent behavioural disorders.

The anti-inflammatory role of Angiotensin-(1-7) in human choriodecidua

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Background: Inflammation has long been implicated as a key mediator in the onset of parturition in both preterm and term births. Tissue renin-angiotensin systems (RASs) can regulate inflammation. The angiotensin converting enzyme 2 (ACE2)/Angiotensin-(1-7)/Mas receptor anti-inflammatory pathway, has been shown to inhibit the pro-inflammatory cytokines interleukin (IL)-6, IL-8 and tumour necrosis factor (TNF)-α levels and enhance the anti-inflammatory cytokine, IL-10, in pancreatic acinar cells.

Aims/Hypothesis: We aimed to investigate how inflammation affects expression of the RAS in term uncomplicated choriodecidua. Furthermore, we aimed to determine whether Ang-(1-7) affects the expression of inflammatory cytokines in the intrauterine tissues.

Methods: Term non-labouring choriodecidual explants were treated with lipopolysaccharide (LPS, 5μg/ml, O55:B5) or AVE0991 (Mas receptor agonist) for 24h and tissue and culture medium was collected for analysis (n=10). Levels of mRNA expression of RAS genes were measured via qPCR. Levels of IL-1β, IL-6, and IL-10 and TNF-α were measured via qPCR and a BD Cytometric Bead Array Human Inflammatory Cytokines Kit.

Results: Treatment with LPS significantly increased the expression of ACE2 ($P<0.0001$) and IL-10 mRNA ($P=0.005$) in choriodecidual explants. Secretion of IL-1β, IL-6 and TNF-α were significantly increased following treatment with LPS, whereas secretion of IL-10 was reduced ($P=0.0001$, 0.0001, 0.0003 and 0.03 respectively). Treatment with the Mas receptor agonist AVE0991 significantly reduced the mRNA expression of pro inflammatory IL-6 and TNF-α (both $P=0.04$). Treatment with AVE0991 also significantly reduced secretion of TNF-α ($P=0.03$).

Conclusions: These data suggest that the ACE2/Ang-(1-7)/MasR may play a role in regulating inflammation within the choriodecidua during pregnancy and thus pose as a novel therapeutic target to prevent inflammation induced preterm birth.

The effect of hypoxia-ischemia, therapeutic hypothermia and gestational age on the pharmacokinetics of exogenous erythropoietin in fetal sheep

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Background: Treatment with high-dose human recombinant erythropoietin (rEPO) has been examined for neuroprotection in preterm infants and full-term infants with hypoxic-ischemic encephalopathy (HIE). However, the pharmacokinetics of high-dose rEPO in neonates are not completely understood.

Aims: To examine the effects of hypoxia-ischemia (HI), therapeutic hypothermia and gestation age on the pharmacokinetics of high-dose rEPO in fetal sheep. To compare the pharmacokinetics of high-dose rEPO in preterm fetal sheep to those reported in the clinical trials in preterm infants.

Methods: Near-term fetal sheep at 0.8 gestation received sham-ischemia (n=5) or cerebral ischemia for 30 minutes followed by treatment with vehicle (n=8), rEPO (n=8) or combined treatment with rEPO and hypothermia (n=8). Preterm fetal sheep at 0.7 gestation received sham-asphyxia (n=1) or complete umbilical cord occlusion for 25 minutes followed by treatment with vehicle (n=8) or rEPO (n=19). rEPO was administered intravenously as repeated bolus injections or as a loading bolus followed by a prolonged continuous infusion. The dose range was 36320 to 256664 IU in preterm and near-term fetal sheep.

Results: The plasma concentrations of rEPO were best described by a pharmacokinetic model that included mixed-order elimination with increasing Vmax after rEPO exposure. There were no significant effects of exposure to HI, therapeutic hypothermia or gestational age on rEPO elimination. Treatment with a bolus injection of 5000 IU/Kg in preterm fetal sheep achieves an area under the curve for rEPO plasma concentration comparable to preterm infants treated with bolus injections of 1000 IU/Kg.

Conclusions: In fetal sheep, rEPO dose adjustment is not required for combined treatment with therapeutic hypothermia, developmental maturation with gestational age or exposure to HI. These data are fundamental for future studies examining neuroprotection with high-dose rEPO treatment.

Invited Speaker: Ex-Newcastle Session

Microvascular tone regulation in the preterm neonate (and beyond!)

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Cardiovascular compromise is associated with poor outcome in the preterm neonate, with gestational age and male sex as independent risk factors for hypotension, developmental injury and death. The microvasculature plays an important role in the development of cardiovascular compromise in the preterm. Our work has aimed to further characterise microvascular changes that occur in the preterm newborn, identify potential windows for therapeutic intervention, explore the mechanisms underlying this dysfunction, and understand the contribution of this early dysregulation to whole-of-life cardiovascular risk following preterm birth.

We have proposed a hypothetical model of gasotransmitter-dependent vasodilatation in the preterm newborn, including a role for oxidative stress and antenatal glucocorticoids exposure in modulating dysregulation. Our findings suggest varying roles for, and interactions of, the three gasotransmitters – allowing for regional and temporal control of blood flow. Our findings also contribute to a better understanding of the mechanisms underlying the male disadvantage (female advantage) following preterm birth.

We are now exploring cardiovascular dysfunction which persists beyond the immediate postnatal period. Using our guinea pig model of preterm birth we are aiming to better define whole-of-life cardiovascular risk following preterm birth by characterising ex-preterm cardiovascular physiology both at rest and in stressed states. The goal of this work is to identify the mechanisms underlying maladaptation, with a view to developing targeted therapeutic interventions to reduce lifetime burden associated with being born too early.

Blood volume decreases in the hours after preterm birth

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Background: Maintenance of blood volume is critical to cardiovascular stability. Early investigations suggested preterm blood volume per kg is higher than in term babies and adults. However, recent studies report lower and highly variable values. We have shown that blood volume of preterm piglets at 5-11h after birth is lower than in term pigs.

Aims/Hypothesis: The aim was to determine blood volume in preterm piglets over the 11h after birth. We hypothesise that preterm piglets are born with a blood volume similar to term piglets but that this decreases in the first 5h after birth.

Methods: Preterm piglets (97/115d; similar to 27wk) were maintained in intensive care for 11h after birth. Blood volume was measured twice in each piglet using labelled dextrans within 15min of birth and at 5h PNA (n=12), or at 5h and 11h PNA (n=8). Blood volume of spontaneously born term piglets was measured at <24h PNA (n=4).

Results: Blood volume in preterm piglets at birth was similar to term piglets (81±13 mL/kg vs 89±4 mL/kg). In the 5h hours after preterm birth, blood volume decreased significantly by 25% to 60±6 mL/kg and this hypovolemia was still present at 11h after birth (57±5 mL/kg). This reduction in blood volume was paralleled by increases in haemoglobin concentrations.

Conclusions: Hypovolemia may be contributing to cardiovascular instability observed in some preterm infants on the first day of life. Methods to detect and effectively treat hypovolemia in preterm infants are essential to the development of effective cardiovascular support.

Changes in cardiac TRPM7 expression during late gestation and transition to extra-uterine life in preterm and term piglets

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Background: Cardiovascular compromise in the first day of life is common in preterm infants. One factor that may contribute to cardiovascular compromise is functional immaturity of the preterm heart due to immature Ca^{2+} handling in the preterm myocardium. TRPM7, a transmembrane cation channel, serves as a novel candidate in regulating intracellular cardiac calcium in the early neonatal period, and may provide an alternative treatment target for cardiovascular compromise.

Aims/Hypothesis: The aim of this study was to determine a) the ontogeny of *TRPM7* expression in neonatal piglet hearts in late gestation, b) the effect of transition to extrauterine life on *TRPM7* expression in preterm and term piglets, and c) any sex specific difference in *TRPM7* expression at birth

Methods: The mRNA expression of *TRPM7* was quantified in the left ventricle of both male and female preterm (91d and 97d) and term (113d) piglet hearts at birth using quantitative real-time PCR. Expression was also determined at 6 hours of postnatal life in preterm (97d) and term (113d) piglets.

Results: *TRPM7* expression at birth was similar in preterm and term infants. In term piglets, expression of *TRPM7* was increased after 6h of extrauterine life. In comparison, expression of *TRPM7* did not change in preterm piglets after 6h of extrauterine life. Male piglets have decreased expression of *TRPM7* at birth compared with female piglets across all gestational age groups.

Conclusions: Upregulation of myocardial TRPM7 may be part of the mature adaptation to extrauterine life. Failure to upregulate TRPM7 in preterm infants may contribute to poor cardiovascular function. Therefore, TRPM7 offers a potential treatment target to improve cardiac function in preterm infants.

Investigation of the Placental Metallome via X-Ray Fluorescence Microscopy: Potential Markers of Placental Health

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Background: Intrauterine growth restriction (IUGR), preterm birth, prolonged pregnancy, and stillbirth are associated with placental dysfunction and have long-term pathological consequences for the offspring, with some evidence that placental health is dependent upon appropriate metal homeostasis. Thus, investigation of the placental metallome is pivotal for understanding the aetiology of gestational morbidities, and Synchrotron technology is an unambiguous method for the identification and quantification of metal ions in biological tissue.

Aims/Hypothesis: This study aimed to elucidate the changes in concentration and distribution of metals in early term, late term, IUGR and stillbirth placentas by Synchrotron X-Ray Fluorescence Microscopy analysis.

Methods: X-Ray Fluorescence Microscopy imaging was used to spatially resolve the distribution and concentration of metals in 24 frozen, unfixed, 60µm placental tissue sections. Experimental groups consisted of 7 early term (37-39 wks), 7 late term (41+ wks), 5 IUGR, and 5 stillbirth pregnancies. Metal concentrations (ppm, parts per million) were measured via GeoPIXE software. Mann-Whitney U test was performed to calculate differences between groups.

Results: Statistically significant differences in elemental concentrations (ppm) were found between different placental groups. K ($p<0.05$), Ca ($p<0.01$), Zn ($p<0.01$) and Mn ($p<0.05$) concentrations were elevated in stillbirth placentas in comparison to term controls; conversely, Ca ($p<0.01$) and Zn ($p<0.01$) concentrations were lower in IUGR placental tissue. Fe ($p<0.05$) and Mn ($p<0.05$) were both elevated in late term placentas, while Cu concentration did not change significantly across placental sets.

Conclusions: The metallome of the placenta varies significantly in different pathological conditions. It is as yet unclear whether this represents differences in the uptake of metals from the maternal circulation or differences in the transport of metals to the fetus or a combination of these mechanisms. It is also unclear whether the changes observed are causative or consequential.

Magnesium sulphate attenuates oligodendrocyte maturation after asphyxia in preterm fetal sheep

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Background: Magnesium sulphate ($MgSO_4$) is widely recommended for preterm neuroprotection. However, to date, follow up to school age has found an apparent lack of long-term clinical benefit. Because of this inconsistency, it remains controversial whether $MgSO_4$ offers sustained neuroprotection. Furthermore, in large animal trials short term analysis of $MgSO_4$ for HIE indicates a significant anti-excitatory effect of $MgSO_4$ on post-asphyxia seizures but a deleterious effect on oligodendrocyte survival.

Aims/Hypothesis: To evaluate the long-term effect of $MgSO_4$ on white and grey matter damage in preterm fetal sheep exposed to asphyxia.

Methods: Chronically instrumented near term fetal sheep (0.7 gestation) were randomly assigned to receive sham occlusion (n=6), i.v. $MgSO_4$ (n=6), or saline (n=6) starting 24 h before asphyxia until 24 h after asphyxia. Sheep were humanely killed 21 days after asphyxia and fetal brains were collected for histopathology.

Results: After 21 days recovery, asphyxia was associated with loss of cortical and striatal neurons ($P<0.05$ vs. sham occlusion), and increased microgliosis, no significant loss of total (Olig-2+) oligodendrocytes but a reduction in mature MBP+ oligodendrocytes ($P<0.05$ vs. sham occlusion). $MgSO_4$ did not affect cortical or striatal neuronal loss or white matter microgliosis. $MgSO_4$ was associated with reduced numbers of all (Olig-2+) oligodendrocytes compared to saline-treated controls ($P<0.05$) in the intragyral and periventricular white matter tracts, and greater loss of MBP+ oligodendrocytes in the periventricular white matter than saline-treated controls ($P<0.05$).

Conclusions: A clinically comparable dose of $MgSO_4$ during acute hypoxia-ischemia in preterm fetal sheep did not improve neuronal survival and was associated with a sustained loss of total and myelinating oligodendrocytes after 21 days recovery. These data suggest that in the setting of preterm HIE, further preclinical testing is needed to ensure safety and best practice regimens.

What about mum? Gut adaptations to maximise nutrient uptake during mouse pregnancy

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Background: Increased food intake during pregnancy is associated with reduced central satiety, the development of leptin resistance and changes in sex hormones. However, even though appropriate maternal nutrition during pregnancy is essential for normal fetal growth and optimal progeny survival and health, pregnancy related adaptations in the gastrointestinal (GI) tract are poorly understood.

Aims/Hypothesis: We therefore characterised changes in body weight, feeding patterns, the satiety signalling of gastric vagal afferents and small intestinal anatomy during murine pregnancy.

Methods: Female C57BL/6J mice (10-12 wk) were mated and randomised to study in early (6.5 d, N=10), mid (12.5 d, N=10) or late (17.5 d, N=11) pregnancy, or used as age matched, non-pregnant controls (metabolic outcomes N=10-12 per group; electrophysiology N=7-9 per group). From mating or equivalent ages, mice were singly housed in Promethion cages to assess weight and behaviour, prior to humane killing and tissue collection at 0700h. The mechanosensitivity of gastric vagal afferents were determined using an *in vitro* single fibre recording preparation, while intestinal tissues underwent histological analyses.

Results: Pregnant mice were heavier from d 6.5 ($P < 0.05$) and ate more during the light phase on d 6.5-7.5 and 12.5-15.5 ($P < 0.05$), predominantly due to increased meal size. Dark phase food intake did not differ between groups. The mechanosensitivity of gastric vagal afferents to stretch (0.5-5 g) decreased as pregnancy progressed ($P < 0.05$). The small intestine was heavier in mid- and late-pregnant mice compared to non-pregnant mice (each $P < 0.001$), and duodenal villi length increased throughout pregnancy ($R = 0.54$).

Conclusions: The progressive reduction in mechanosensitivity of gastric vagal afferents, and increase in intestinal surface area, during pregnancy are likely to promote food intake, and nutrient absorption, respectively. Our findings provide strong evidence that the GI tract adapts to ensure the energy demands of pregnancy are met for healthy fetal growth.

In utero Δ9-tetrahydrocannabinol exposure alters endocrine pancreatic development in female offspring leading to impaired glucose tolerance in adulthood

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Introduction: Recent reports indicate that 1 in 5 pregnant mothers aged 18-24 years in North America use *cannabis*. This is concerning given that *in utero* exposure to Δ9-tetrahydrocannabinol (∆9-THC), the main psychoactive component in *cannabis*, causes placental insufficiency and intrauterine growth restriction (IUGR). ∆9-THC also activates the cannabinoid receptor CB1R in pancreatic β-cells to alter cell cycle progression and stimulate apoptosis.

Aims/Hypothesis: Therefore, this study examined the effects of *in utero* ∆9-THC exposure on endocrine pancreatic development, and its impact on glucose homeostasis in adulthood.

Methods: Pregnant Wistar rats were treated with daily intraperitoneal injections of either 3 mg/kg ∆9-THC or vehicle from gestational day 5.5 to birth. Male and female offspring were sacrificed at postnatal day 21 (PND21) or 6 months of age. Pancreata were harvested, fixed, embedded in paraffin, sectioned, and immunohistochemistry used for morphometric analysis. Glucose tolerance was assessed at 5 months. Ins-1E cells were employed to determine the direct effects of ∆9-THC on markers of β-cell development.

Results: Maternal exposure to ∆9-THC led to symmetrical IUGR and reduced pancreatic weight without impacting litter size, gestational length, or maternal weight gain. Endocrine pancreatic development was altered exclusively in ∆9-THC-exposed female offspring, with these animals exhibiting reduced β-cell mass at PND21 and 6 months of age, as well as reduced α-cell mass and total islet density at 6 months. Glucose tolerance was impaired in these offspring at 5 months of age. In Ins-1E cells, ∆9-THC markedly suppressed the steady state levels of Pdx-1 and insulin mRNA, both of which are hallmarks of β-cell differentiation and function.

Conclusions: *In-utero* ∆9-THC exposure leads to striking sexual dimorphism in endocrine pancreatic development and glucose tolerance in adulthood. While the mechanism remains to be fully elucidated, these results raise concern over the metabolic health of offspring, particularly females, exposed to *cannabis in utero.*

Invited Speaker: Ex-Newcastle Session

Antibiotic Treatment of Placental and Fetal Inflammation in a Non-Human Primate Model of Intra-Amniotic *Ureaplasma* Infection

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Background: Ureaplasma parvum is a prevalent cause of intrauterine infection that is associated with chorioamnionitis, preterm birth, and the fetal inflammatory response syndrome. We have previously demonstrated that in a non-human primate model, maternal treatment for intra-amniotic Ureaplasma infection clears microbes from the amniotic fluid, prolongs gestation, and reduces fetal injury.

Aims: To examine whether antibiotic treatment alone (Azithromycin) or in combination with anti-inflammatory agents would diminish placental inflammation and histological signs of chorioamnionitis.

Methods: Chronically catheterized pregnant rhesus monkeys were assigned to control (n=7), intra-amniotic infection (IAI, n=6), Azithromycin alone (12.5 mg/kg q 12h for 10 days; AZI, n=6) or AZI in combination with dexamethasone (4 mg/kg/d, IV for 4d) and indomethacin (100 mg/d PO for 5d; ADI, n=7). Animals in the IAI, AZI and ADI groups received intra-amniotic inoculation with U.parvum (serovar 1) at 127d gestation (term=167d). Antibiotic/anti-inflammatory treatments began 6-8 days after U. parvum infection coinciding with increased uterine contractions/cervical effacement. Placentas and membranes were serially sectioned and scored for histologic evidence of chorioamnionitis. Placental expression of inflammatory genes was determined by Gene Immunearray and RT-qPCR. Statistical significance ($p<0.05$) was assessed by Fisher's exact test and ANOVA.

Results: Maternal Azithromycin and anti-inflammatory treatment was associated with a decrease in the severity of histologic chorioamnionitis and the frequency of funisitis when compared to the infection group ($p<0.05$). Gene expression of IL1B, TNFA and CCL3 was increased in the placentas from the IAI group ($p<0.05$). Azithromycin combined with anti-inflammatory agents appeared to be more effective in reducing inflammatory gene expression, with greater reductions in TNFA and CCL3 expression than AZI alone ($p<0.05$). Intrauterine Ureaplasma infection also increased umbilical artery pulsatility index measured by Doppler ultrasound and evidence of fetal neuroinflammation, both of which were improved following antibiotic treatment.

Conclusions: Our findings indicate that maternal antibiotic therapy for infection-associated preterm labor (alone or in combination with anti-inflammatory agents) substantially reduces functional and histological evidence of placental and fetal inflammation. These findings have significance for the continued development of therapies for intrauterine infection and inflammation in order to prevent preterm labor and potential fetal and neonatal negative sequelae of exposure to intrauterine infection and inflammation.

IL-1RA reduces neuroinflammation and improves oligodendrocyte survival in near-term fetal sheep

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Background: Antenatal exposure to systemic inflammation/infection is a major risk factor for injury and long-term disability in late preterm/term infants. The efficacy of therapeutic hypothermia in infants exposed to systemic inflammation/infection may be impaired in babies exposed to gram negative infection. Therefore, it is vital to establish a therapeutic intervention for infants that are not responsive to current hypothermia protocols.

Aims/Hypothesis: To test the hypothesis that administration of an interleukin-1 receptor antagonist (IL-1RA) would attenuate lipopolysaccharide (LPS)-induced neuroinflammation and injury in the white matter of near-term fetal sheep.

Methods: Chronically instrumented near term fetal sheep (0.85 gestation) were randomly assigned to receive either saline (control; n=9), lipopolysaccharide (LPS) (300 ng/24 h then doubled every 24 h for 2 days; n=7), or LPS+IL-1RA (15mg/kg 1 hour after LPS exposure; n=5). Four days after starting LPS/saline infusion, fetal brains were collected for histopathology.

Results: LPS infusions caused transient reductions in mean arterial blood pressure, and increased carotid artery perfusion and fetal heart rate ($P<0.05$ vs. control). EEG power was reduced in LPS exposed fetuses between 72 and 96 hours ($P<0.05$ vs. control). LPS exposure increased IL-1β immunoreactivity and microglial number, and reduced astrocyte and total (Olig-2+) oligodendrocyte survival in the intragyral and periventricular white matter. IL-1RA improved recovery of EEG power, reduced microgliosis, and improved oligodendrocyte survival in the intragyral and periventricular white matter tracts ($P<0.05$, IL-1RA+LPS vs. LPS), but did not significantly improve astrocyte survival ($P<0.06$, IL-1RA+LPS vs. LPS).

Conclusions: IL-1RA administration improved recovery of brain activity, reduced neuroinflammation and improved oligodendrocyte survival in the white matter of LPS-exposed fetal sheep. This suggests that IL- 1 inhibition could be a promising therapeutic intervention to reduce neuroinflammation and improve white matter development in infants exposed to inflammation around the time of birth.

Maternal sleep in late gestation pregnancy

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Background: Sleep is composed of ultradian (90-120min) cycles between rapid eye movement (REM) sleep and non-REM (NREM) sleep and in different proportions of time as the night progresses. These different phases of sleep confer different physiological benefits. Pregnancy is associated with impaired sleep, with insomnia and reduced REM. However, sleep architecture in complicated pregnancies, such as obesity, is not well understood.

Aims/Hypothesis: To characterise maternal sleep architecture in normal and complicated pregnancies.

Methods: Pregnant women in late gestation (mean ~36 weeks) were recruited from normal (n=32) and complicated pregnancies; obesity (n=31), reduced fetal movement (n=32), small for gestational age fetuses (n=36), and maternal supine hypotension (n=26), to complete an overnight in-home modified level II polysomnography study. A three-channel EEG recording was scored for wake, 3 stages of NREM (N1/N2/N3) and REM.

Results: Preliminary analysis showed no differences between groups. General structure of sleep was preserved in all pregnancies: NREM predominant in the first sleep cycle and increasing REM with successive cycles. However, the average sleep cycle length was reduced compared with adult non-pregnant sleep (~60min vs ~90min). The proportion of REM and NREM, particularly N3, appears to be preserved in cycle one, but thereafter more time was spent in REM. Thus, while sleep duration was less, the total amount of time spent in REM was preserved at the expense of NREM, and in particular N3.

Conclusions: Our study confirms that sleep during third trimester pregnancy is disturbed and pregnancy complications such as obesity don't change this. We have demonstrated that REM is preserved at the expense of NREM, underscoring the importance of REM sleep to our daily neural function. NREM is usually the dominant form of sleep, vital for rest and energy replenishment, and N3 is key phase for rest and repair. Reduced NREM sleep in pregnancy likely contributes to fatigue during pregnancy. Further work is needed to examine specific sleep stages in each cycle and to examine differences within groups, including sleep disordered breathing.

The evolution of neuronal injury after hypoxia-ischaemia in preterm fetal sheep

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Background: Many cases of cerebral palsy and impaired neurodevelopment are associated with hypoxia-ischemia (HI) before birth, during preterm fetal development. HI leads to long-term impairment of myelination and neuroinflammation, both of which likely contribute to poor connectivity. Our previous study demonstrated that significant evolving injury is observed weeks after an HI insult with diffuse white matter injury at three and seven days, evolving into a spectrum of severe white matter degeneration including cystic white matter lesions, white matter atrophy, and ventriculomegaly by 21 days post-HI. It is unclear what impact this late onset injury has on the associated neurons.

Aims/Hypothesis: To determine the long term outcome of neuronal injury after an acute HI insult in preterm fetal sheep.

Methods: Chronically instrumented fetal sheep (0.7 gestation) underwent sham HI or HI induced by 25 min of umbilical cord occlusion. Fetal brains were processed for histology post-HI at 3 days (n=9, sham n=12), 7 days (n=8, sham n=8), 14 days (n=9, sham n=10) and 21 days (n=9, sham n=10).

Results: We observed consistent acute neuronal loss throughout the 21 days of recovery. Asphyxia was associated with a marked reduction in total cortical area at 21 days recovery, from 92.1 ± 30.6 mm^2 to 73.9 ± 35.7 mm^2 ($P = 0.05$). Preliminary results suggest a significant reduction in thalamic area in HI brains at 7, 14 and 21 days. A consistent reduction in neuronal densities was also observed in both the caudate nucleus and putamen of the striatum from 3 to 21 days recovery.

Conclusions: In contrast to the slow evolution of white matter injury observed, preliminary results from this study suggests that subcortical neuronal injury is largely established in the first 3 days after the insult, and from then on no endogenous recovery in neuronal density occurs.

Hypothermia attenuates KCC2 expression after neonatal HI in the pig

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Background: Current clinical treatment for perinatal hypoxia-ischaemia (HI) is limited to therapeutic hypothermia (TH), and symptomatic treatment of neonatal seizures. GABA agonists such as barbiturates and benzodiazepines are used to treat neonatal seizures. In the immature brain GABAergic neurotransmission is excitatory, and contributes to excitotoxic damage and seizures following neonatal HI. The modulation of GABA action from immature excitation to mature inhibition is in part, dependent on the expression of the potassium chloride cotransporter 2 (KCC2) protein.

Aims: We aimed to investigate the expression of KCC2 in the newborn pig brain after HI, with and without TH.

Methods: Animals (postnatal day 1, P1, n=16) were exposed to 30min hypoxia with systemic hypotension, followed by 24h of TH (n=8) or recovery under normothermic (NT, n=8) conditions. ^{1}H-MRS was performed at 72h post-HI, prior to euthanasia (P4). Sham animals were housed in the same facility and underwent MRS imaging at P4, before euthanasia. KCC2 protein levels were determined using Western blot, and cellular distribution observed by immunofluorescence.

Results: HI animals treated with TH had significantly lower MRS lactate ratios compared with HI-NT animals, indicating greater neuroprotection. MRS ratios correlated significantly with parietal cortex KCC2 expression. HI-HT had significantly higher levels of KCC2, in parietal cortex and cerebellum, when compared with sham and HI-NT. HI-NT and sham animals had no difference in KCC2 protein expression levels.

Conclusions: KCC2 has been reported to play a role in neuroprotection, and the upregulation we observed after TH may in part explain the observed reduction in HI-induced neuropathology. KCC2 promotes the balance of inhibition and excitation, potentially alleviating the excitotoxic burden of neonatal HI.

Cleavage of the soluble (pro)renin receptor (s(P)RR) in the placenta

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Background: The extracellular domain of full-length (pro)renin receptor ((P)RR) can be cleaved and secreted as soluble (P)RR (s(P)RR). This 28 kDa s(P)RR is detected in plasma and urine. s(P)RR is elevated in the plasma of pregnant women with preeclampsia, fetal growth restriction and gestational diabetes mellitus, implicating it as an important biomarker for disease. However, the source and cleavage of s(P)RR in pregnancy is unknown. The placental syncytiotrophoblast cell layer is responsible for the secretion of hormones and waste products into the maternal circulation. The pro-protein convertases (PCs), FURIN and site 1 protease (MBTPS1), are known to cleave the s(P)RR in Chinese hamster ovary cells (Cousin et. al 2009; Nakagawa et. al 2017).

Aims/Hypothesis: We hypothesise that s(P)RR is secreted by the syncytiotrophoblast layer of the placenta during pregnancy, and that FURIN and/or MBTPS1 is responsible for its cleavage in the placenta.

Methods: Primary trophoblast cells were isolated from term human placentae and treated with either *FURIN* siRNA or negative control siRNA. Separately, cells were treated with the FURIN inhibitor, DEC-RVKR-CMK, an MBTPS1 inhibitor, PF 429242, or vehicle. Trophoblasts were left to spontaneously syncytialise before cells and supernatant were collected and levels of s(P)RR measured. N=4 placentae in triplicate.

Results: s(P)RR was secreted by primary trophoblast cells and both intra and extra-cellular s(P)RR levels decreased with syncytialisation (P=0.01 and P=0.02, respectively). *FURIN* siRNA successfully knocked down FURIN mRNA expression by 66% and protein levels by 58% (both P<0.0001), however it had no effect on intra or extra-cellular s(P)RR levels. DEC-RVKR-CMK significantly decreased extracellular (P=0.02), but not intracellular, s(P)RR protein levels. PF 429242 had no effect.

Conclusions: We are the first to show that s(P)RR is secreted by the placenta and that s(P)RR levels decrease with syncytialisation. DEC-RVKR-CMK, a broad inhibitor of all PCs, decreased extracellular s(P)RR levels, but *FURIN* siRNA knockdown and the Site1 protease inhibitor had no effect. These findings suggest that a PC other than FURIN or MBTPS1 is responsible for s(P)RR cleavage in the placenta.

The effects of *in-utero* hypoxia and creatine treatment on the fetal hippocampus

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Background: Acute *in-utero* fetal hypoxia has region-specific effects on the developing brain, particularly the hippocampus. We hypothesise that injury to the hippocampus may be linked to mitochondrial dysfunction due to the high metabolic activity of this brain region. Creatine is a naturally occurring dietary metabolite that acts as a spatial and temporal energy buffer by generating ATP anaerobically. It is neuroprotective against neonatal and adult brain trauma by maintaining mitochondrial integrity but is yet to be investigated in the fetal context.

Aims/Hypothesis: To characterize hypoxic-mediated mitochondrial injury in the hippocampus and assess whether creatine supplementation protects the fetal brain by improving mitochondrial function.

Methods: At 118 days of gestation (term = 147), surgically prepared singleton fetuses of 28 pregnant ewes were randomly allocated to receive an intravenous infusion of either creatine (6mg/kg/h) or saline. After 9 days of infusion, fetuses were subjected to 10 mins of severe fetal hypoxia by complete occlusion of the umbilical cord [UCO] (n=8 creatine, n=9 saline infused). Control fetuses (n=6 creatine, n=7 saline infused) did not receive UCO. At 72 h post-injury the hippocampus was collected for assessment of mitochondrial function, cell death, creatine content and changes in gene expression. Data was analysed by Two-Way ANOVA with Turkey's multiple comparisons.

Results: Hippocampal mitochondrial respiration was decreased ($p<0.05$) at 72 h after UCO; this outcome was unaffected by creatine pre-treatment. Increased cell death ($p=0.02$), cytochrome *c* release ($p<0.01$) and pro-apoptotic BAK expression ($p<0.05$) were also observed in the UCO-saline fetuses relative to controls; creatine pre-treatment decreased cytochrome *c* release ($p<0.05$), increased anti-apoptotic *BCL2* mRNA ($p<0.01$), and decreased stress-markers *CAS3* ($p=0.01$) and *CRSL1* ($p<0.05$).

Conclusions: Mitochondria in the fetal hippocampus are vulnerable to acute hypoxic injury *in utero*. Creatine pre-treatment ameliorated some markers of injury in this region, but did not rescue mitochondrial function.

Expanded Umbilical Cord Blood Cell Therapy for Hypoxic Ischemic Brain Injury

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Background: Hypoxic ischemic (HI) insults during pregnancy and birth can result in long-term neurodevelopmental disorders, such as cerebral palsy. We have previously shown that multiple doses of human umbilical cord blood (UCB) cells are needed to effectively modulate long-term neuropathology and behavioural outcomes. To achieve the number of cells required to administer multiple doses it is likely that UCB expansion may be required. In this study, we investigated the long-term efficacy of expanded UCB-derived CD34+ cells, compared to unexpanded UCB. We hypothesised that multiple doses of expanded UCB cells will reduce long-term neuropathology and behaviour as effectively as unexpanded UCB.

Methods: HI injury was induced in postnatal day (P)10 rats by left carotid artery ligation, followed by 90min hypoxia (8% O_2). Pups were administered 1 dose (P11), or 3 doses (P11, 13, 20) of unexpanded UCB cells or expanded UCB cells (1 million cells/dose). Rats were monitored until P50 and behavioural testing was carried out over this period. On P50, brains and blood were collected for immunohistochemistry, molecular and protein analysis.

Results: To date, 70% of the animals have been completed for this study, and we have assessed short and long-term behavioural outcomes. At P30 we observed significant limb asymmetry in the HI group, as well as the single dose of expanded cells group, while the sham group and other cell treated groups showed no limb asymmetry deficits. We have analysed brain and body weight from the completed animals. Preliminary results show that the expanded UCB cells confer no adverse effects, and thus were shown to be safe in this model of brain injury. Next, we will assess neuropathology including analysis of tissue loss, neuroinflammation and changes to neuronal populations.

A Novel Hydrogel Therapy for Neonatal Stroke

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Background: Neonatal stroke (NS) leads to permanent neurological deficits. The delay to diagnosis is on average 24 h; therefore, appropriate treatments for NS need to be effective if given even after 24 h or more.

Aims/Hypothesis: 1) to optimise the temporal release profile of therapeutic peptides from a biocompatible hydrogel to be injected directly into stroke tissue; 2) to study photothrombotic-NS in rat pups for in vivo testing of hydrogel therapy; and 3) to establish the safety and efficacy of delivering our hydrogel into the core of the lesion at 24 hours post-NS.

Methods: Release of the growth factors IGF1 and Reg3 from a functionalised hydrogel (Fmoc-DIKVAV+fucoidan) was determined over 20 days. NS in P10 rat pups was induced by 10 min exposure to a 1mm diameter beam of 565 nm light through the skull and into the cortex, after i.p injection of Rose Bengal (25mg/kg). Lesion volume was quantified after 1 or 7 days by H&E staining, and by IHC for glial-fibrillary acidic protein (GFAP) and Iba-1 gliosis. Locomotion (Open Field) and forelimb strength (wire-hang test) testing was done at +1 and +6 days post-NS, and compared to age-matched controls pups (stroke procedure withheld).

Results: The *in vitro* studies revealed differential time-dependent release from the hydrogel of IGF1 (8%/24 h; 1.3–3.5%/day over 14 day), and Reg3 (~0.06%/24h; 0.013%–0.031%/day over 20 days). Cortical lesion volume was 3.2± 0.30mm^3 (mean ± SD, n=4) at 24 hours post-NS, with discreet glial borders and >90% in the primary motor cortex. Preliminary behavioural testing shows a reduction in open-field activity 1 and 6-days post-NS.

Conclusion: We have a biocompatible hydrogel that can deliver multiple drugs in a temporally-regulated manner. Also, we have described a non-invasive and reproducible model of NS in which to perform rapid neurotherapeutic testing.

Gestational age-dependent haemodynamic response to thermal stress in juvenile guinea pigs

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Background: Globally, there is an increase in cardiovascular morbidity and mortality associated with heat waves. It is therefore important to understand the cardiovascular tolerance of vulnerable populations to heat stress. Ex-preterms, one such vulnerable population, have previously shown poor tolerance to cardiovascular stress testing, due to persistent changes in cardiovascular structure and function. Thus, those born preterm may be less able to tolerate cardiovascular challenges imposed by increased ambient temperatures.

Aims/Hypothesis: We aimed to investigate the cardiovascular response to heat stress testing. We hypothesised that in juvenile guinea pigs **(**36.5 ± 1.5 days; equivalent to 8-10 y/o children) those born preterm (preterm, gestational age d62; term, d69) would have significantly impaired homeostatic responses to heating.

Methods: Sedated guinea pigs (0.8% isoflurane, 70% N_2O) were incrementally heated 1^{o}C/min to 44^{o}C from 33-34^{o}C, using a water-perfused wrap, until rectal temperature reached 41.5^{o}C then recovered at 35^{o}C for 30 min. Continuous physiological monitoring of ECG, blood pressure, microvascular flow at ear and interscapular skin sites, respiration rate as well as rectal and skin temperature was performed. Capillary blood gas measures were taken pre, and post (Post-10min, Post 3-hr, Post-24hr) challenge.

Results: Thermal stress tests were completed in n=7/sex/GA. Core temperature increased to 41.5 ^{o}C across 35-40 min of challenge. Preliminary results indicate that compared terms, preterms have diminished ear perfusion during thermal stress and altered acid base balance, pH and lactate production during post-challenge recovery. Heart rate and respiratory rate responses were similar between groups throughout.

Conclusions: The capacity to physiologically buffer the effects of rising core body temperature determines survivability during thermal extremes. Animals born preterm had sexually dimorphic responses to heating. The mechanisms underpinning the altered central and peripheral responses are being examined to determine the relative weighting of factors contributing to the preterm-associated cardiovascular morbidity.

Immunosuppression: Effects in Neonatal Rat Pups following Brain Injury

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Background: Each year, ~60 babies in Australia are diagnosed with perinatal stroke, which leads to significant neurological deficits and has no specific treatment. Neural stem cells (NSCs) have the potential to engraft into the neonatal brain and replace lost neurons following stroke. However, NSCs possess MHC class I and II antigens which may lead to rejection in the brain. Immunosuppression could promote NSC survival, increasing engraftment and efficacy of NSCs, but the effect of immunosuppression on the neonate and the injured brain is not known.

Aims/Hypothesis: To determine the safety and efficacy of an immunosuppressive drug (tacrolimus), and to determine whether tacrolimus modulates brain injury following HI surgery in neonatal rats.

Methods: To determine tacrolimus safety and efficacy, PND8 Sprague-Dawley rat pups received daily injections of the immunosuppressive drug tacrolimus (i.p. 0.25, 0.5 or 1 mg/kg/day) or PBS. Pups were culled at either PND11 or PND50 and splenocytes were stimulated with PMA+ionomycin and supernatant was collected to determine the cytokine response. PND50 pups underwent motor and cognitive testing, with the rotarod and novel object recognition tests. To determine the effect of tacrolimus following HI surgery, PND8 pups received daily injections of tacrolimus (0.1 or 0.25mg/kg/day) or PBS. On PND 10, pups underwent carotid artery ligation and were subsequently exposed to 8% oxygen for 90 minutes to induce brain injury. At PND17 pups were euthanised and brains were collected.

Results: All three doses of tacrolimus significantly decreased the production of interleukin-4 ($P<0.002$ for all doses). Tacrolimus did not affect rat pup cognitive or motor function, but the two higher doses adversely affected pup weight and survival. Analysis for the tacrolimus and HI surgery rat pups are ongoing.

Conclusions: All three doses of tacrolimus suppressed the neonatal immune system. Results from ongoing and future studies will provide essential information on how to best translate neural stem cells into the clinic.

Wave power analysis of pulmonary arterial blood pressure and flow changes after immediate cord clamping with a non-asphyxial or asphyxial cord clamp-to-ventilation interval at birth in preterm lambs

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Background: Immediate cord clamping (ICC) prior to ventilation at birth is associated with marked changes in pulmonary arterial (PA) blood pressure and flow waveforms, particularly if a brief asphyxial state develops in the cord clamp-to-ventilation (CC-V) interval, but physiological implications of these changes are not fully defined.

Aim: To define right ventricular (RV) and pulmonary contributions/interactions during changes in PA blood pressure and flow in the birth transition after a non-asphyxial or asphyxial CC-V interval via wave power analysis.

Methods: High-fidelity left PA blood pressure (P) and flow (Q) were measured in anaesthetized preterm fetal lambs (gestation 128 ± 2 d) subjected to a ~40 sec non-asphyxial (40sCC, n=8) or ~90 sec asphyxial (90sCC, n=8) CC-V interval after ICC, prior to mechanical ventilation for 30 min after birth. Total PA flow was obtained as the product of left PA flow and post-mortem total-to-left lung weight ratio. PA wave power (WP) was calculated as the product of P and Q rates of change, followed by standard separation into forward and backward waves.

Results: Changes in PA pressures, flow and WP were relatively minor after ICC in the 40sCC group. However, emergence of an asphyxial state after ICC in the 90sCC group was accompanied by 1) marked decreases in PA mean/pulsatile pressures and flow, 2) decreases in WP of an RV-derived, early-systolic, flow-promoting PA forward compression wave (FCW), and a mid-systolic flow-decreasing PA backward compression wave (BCW) arising via pulmonary reflection of the FCW, and 3) a rise in the PA BCW/FCW WP ratio, which was indicative of pulmonary vasoconstriction. After onset of ventilation, surges in FCW and BCW WP in the 90sCC group were pronounced compared with the 40sCC group, consistent with augmented RV contractility, but the BCW/FCW WP ratio dropped markedly in both groups, indicative of pulmonary vasodilation.

Conclusions: Alterations in PA WP accompanying PA pressure and flow changes after ICC with non-asphyxial or asphyxial CC-V intervals during preterm birth provide valuable information about RV-pulmonary interactions.

Project proposal: The effect of neonatal seizures and therapeutic hypothermia following HI on phosphorylated KCC2 expression.

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Background: Hypoxic ischemic encephalopathy (HIE) resulting from disrupted oxygen supply to the brain during the perinatal period, is associated with neurodevelopmental delays, disability, and mortality. Therapeutic hypothermia (TH) is currently the only clinical treatment administered for moderate HIE. HIE is also the leading cause of neonatal seizures, which are associated with exacerbated neuronal injury. Over-excitation or excitotoxicity is a key pathway of HI injury in the newborn brain. In the mature brain GABA is the major inhibitory neurotransmitter, however due to high intracellular chloride (Cl^-) during development, GABA acts as an excitatory neurotransmitter, thus the developing brain is at greater risk of excitotoxic mediated damage. During development intracellular Cl^- is regulated by changes in expression of the cation cotransporters NKCC1, (Cl^- import) and KCC2 (Cl^- extrusion) which in turn modulates GABAergic function. Developmental upregulation of KCC2 reverses the Cl^- gradient leading to a switch in GABAergic function from excitation to inhibition. Phosphorylation of KCC2 at Serine940 residue (pKCC2-S940) is integral to the function of KCC2. Investigating changes to pKCC2-S940 in a preclinical HI model with TH, will help understanding of injury mechanisms and identify potential avenues for neuroprotection.

Aim/hypothesis: We aim to investigate the level of protein expression of pKCC2-S940 in the HI neonatal pig. We hypothesise that TH after HI will attenuate pKCC2-S940 protein expression and the cellular localisation to the membrane.

Methods: Parietal cortex and cerebellum samples of HI animals with and without TH will be used. Pre-recorded physiological and histological data including arterial blood gases (ABG), aEEG, neurobehavioral score, H&E, and MR spectroscopy will be used for injury analysis. Western blot and immunofluorescence will be used to analyse protein expression and cellular localisation of pKCC2-S940. Further analysis investigating the impact of seizure burden on pKCC2-S940 expression will be performed, by comparing animals that developed seizures post-HI with those that did not.

Maternal choline supplementation increases fetal body weight and placental:fetal ratio in a rat model of periconceptional alcohol exposure

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Background: Given that ~80% of Australian women of reproductive age consume alcohol and ~50% of pregnancies are unplanned, exposure of the early embryo to alcohol is likely commonplace. To explore the effects of alcohol exclusively around conception, we have developed a unique rat model of periconceptional ethanol exposure (PCE), which results in fetal growth restriction, placental abnormalities and adult offspring disease (1,2). Choline is a micronutrient and methyl donor essential for development but levels are reduced by alcohol. Previous studies have shown maternal choline supplementation attenuates some effects of prenatal alcohol throughout pregnancy (3). We have utilised our rat PCE model to examine the efficacy of choline supplementation from mid-gestation, which corresponds with the timing of pregnancy recognition in women.

Aims/Hypothesis: We hypothesised that mid-gestational choline supplementation would attenuate the fetal growth restriction and restore any alterations in placental global methylation/gene expression caused by PCE.

Methods: Sprague-Dawley dams were treated with 12.5% v/v EtOH (PCE) or 0% v/v EtOH (Control) liquid diet from 4 days prior to mating until embryonic day 4 (E4). A subset of dams were given choline fortified chow from E10-E20. At E20, maternal and fetal plasma were analysed for choline; placental DNA and RNA were extracted for DNA methylation and qPCR analysis of choline transporters (*Ctl1*, *Ctl2*) and insulin-like growth factor 2 (*Igf2*).

Results: PCE reduced fetal weight, fetal plasma choline concentration, and placental-to-fetal ratio with no effect on expression of placental *Ctl1*, *Ctl2* and *Igf2*. Maternal and fetal plasma choline were significantly higher in all choline supplemented groups. Increased plasma choline was associated with increased fetal weight, placental ratio and expression of placental *Ctl1*, *Ctl2* and *Igf2*. Plasma choline was inversely related to global DNA methylation in the placenta, and this was further exacerbated by PCE in female placentas only.

Conclusions: Increased choline attenuated PCE-induced fetal growth restriction and improved placental-to-fetal ratio. As choline is a methyl donor, it may exert these effects at least in part, by altering DNA methylation within the placenta.

(1) Gardebjer, E.M., et al., Periconceptional alcohol consumption causes fetal growth restriction and increases glycogen accumulation in the late gestation rat placenta. Placenta, 2014. 35:50-57.
(2) Gardebjer, E.M., et al., Maternal alcohol intake around the time of conception causes glucose intolerance and insulin insensitivity in rat offspring, which is exacerbated by a postnatal high-fat diet. FASEBJ, 2015. 29:2690-701.
(3) Akison L.K., et al., Effect of choline supplementation on neurological, cognitive and behavioral outcomes in offspring arising from alcohol exposure during development: A quantitative analysis of clinical and preclinical studies. Alcoholism: Clinical and Experimental Research, 2018. 42:1591-1611.

Validating burst measures of the EEG for assessing changes in brain function due to IVH in preterm infants

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Background: The EEG is a useful tool for brain monitoring in preterm infants. The EEG is, however, a complex signal that can be difficult to interpret. We have developed several summary measures of the EEG that simplify its interpretation and reveal important information on brain function. These measures are based on the accumulated behaviour of bursting in the EEG. In this study, we validate these measures on an independent dataset of EEG recordings from preterm infants.

Aims/Hypothesis: To validate summary measures of EEG for detecting IVH in preterm infants.

Methods: EEG recordings were collected from 54 preterm infants born before 28 weeks gestational age (GA: range 24.3 to 28 weeks) within the first week of life. EEG recordings were segmented in 1 h epochs on which thirteen measures of bursting behaviour were calculated. Measures were averaged across EEG channels (F3-C3, F4-C4) and across all 1 h epochs in a recording (mean 46 ± 19 h). IVH was identified using cranial ultrasound measurements taken at 24, 48 and 72 h and were graded as no IVH, grade I, II or III. Differences in bursts measures between infants with no IVH, grade I and grade II/III were evaluated with a one-way ANOVA (Bonferroni corrected p-values were used to account for 13 measure comparisons). Post hoc analyses were performed using Tukey's HSD test.

Results: The previously identified measure of burst sharpness was significantly different between groups ($F = 11.4$; $p < 0.01$) with a significant decrease between no IVH and IVH I ($p<0.01$) and no IVH and IVH II/III ($p<0.001$). In addition, to these previously studied measures, we also found significant differences in the coefficient of variation of burst duration, minimum burst area, and the slope and intercept of a linear fit to burst area versus burst duration ($p = 0.01$, $p = 0.02$, $p = 0.02$, and $p < 0.01$, respectively).

Conclusions: Burst measures reflect clear changes in the preterm EEG that are associated with IVH. Innovative analyses improve the diagnostic yield of preterm EEG.

The Role of Plasma Angiotensin Converting Enzyme 2 (ACE2)/ Angiotensin-(ANG) (1-7) In Pregnancy Complication: Preeclampsia and Small for Gestational Age

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Background: Preeclampsia (PE) and small for gestational age (SGA) are common pregnancy complications and remain a major health burden to both mother and fetus. Nevertheless, the pathophysiological causes of PE and SGA are not very well understood or documented. Early detection and identification of precise blood biomarkers would enable early interventions to prevent these. During pregnancy, the renin-angiotensin system (RAS) plays significant roles in regulating maternal blood pressure. An imbalance in RAS peptides may contribute to the pathophysiology of PE and SGA.

Aims/Hypothesis: We aimed to examine maternal levels of angiotensin (Ang) peptides and levels/activity of Ang enzymes across gestation and in PE and SGA pregnancies.

Methods: Plasma samples were collected from non-pregnant women (n=10) as well as from women with uncomplicated pregnancies (n=80), SGA (n=25) or PE (n=14) across gestation (13-36 weeks).

Results: Plasma ACE and ACE2 levels as well as ACE2 activity were significantly higher in healthy pregnant women compared with non-pregnant women ($p<0.05$) and remained high throughout gestation with no change in NEP. Ang-(1-7) was also increased in pregnant women compared with non-pregnant women, whereas Ang II was not changed across gestation; thus, Ang II/Ang-(1-7) was not changed. Women with SGA had levels of ACE, ACE2, NEP, Ang II, Ang-(1-7) and ACE2 activity that were similar to healthy pregnant women. On the other hand, ACE and ACE2 levels and ACE2 activity were decreased in women with PE compared with pregnant women.

Conclusions: These studies show for the first time that plasma ACE2 levels and activity increase in pregnancy. ACE2 may play a role in the production of Ang-(1-7), thus activating the vasodilator RAS pathway in pregnancy.

Temporal evolution of inflammation following neonatal hypoxic-ischemic encephalopathy

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Background: Perinatal hypoxic/ischemic (HI) brain injury is a common cause of neurodevelopmental outcomes such as cerebral palsy. A number of pathological processes are initiated following HI, of particular interest to the present study is the expression of pro- and anti-inflammatory mediators which play an important role in the severity of brain injury. These mediators also influence blood brain barrier (BBB) disruption making the brain more susceptible to injury. While the influence of these cytokines and chemokines in progression of HI brain injury is well studied, how the expression levels of these evolve over time has not been studied in detail. Understanding the time course of these mediators may aid in advancement of treatments to improve neurodevelopmental outcomes of neonates after HI injury.

Aims/Hypothesis: To observe the evolution of inflammatory mediators following HI brain injury in a neonate pig model.

Methods: Newborn piglets (<24 h after birth) were used for this study. Hypoxia was induced by decreasing FiO_2 to 4% and adjusted to achieve aEEG peak amplitude <5 μV and hypotension <30 mmHg for the final 10 minutes of a 30 minute HI period. Piglets were recovered from hypoxia by returning inspired O_2 to 21% and survived to 2, 8, 12, 24 h, or 72 h post-HI. Brains were then extracted for histological analysis. Analysis included: neuropathological assessment with haematoxylin and eosin, NeuN labelling (neurons), Fluoro Jade-C (degenerating neurons), cleaved- caspase 3 (apoptotic cells), GFAP (astrocytes), and Iba-1 (microglia). qPCR were performed for markers of inflammation IL-1β, TGFβ, COX2, TNFα, CXCL10, IL-6, IL-8, CCR5.

Results: Preliminary results show early upregulation of pro- and anti-inflammatory markers at 1 hour post-HI compared with sham animals. At 4h post-HI, pro-inflammatory markers were further elevated while there was a down regulation of several anti-inflammatory markers. Morphological examination showed early increases in degenerating neurons, decreased coverage of astrocytes and activation of microglia.

Conclusions: Our early data indicate HI injury results in rapid upregulation of pro-inflammatory markers in the neonatal brain. Our future studies will extend the current preliminary work and understand how brain inflammation relates to systemic inflammation.

Prophylactic creatine alters cerebral oxidative and metabolic stress responses following acute global hypoxia-ischemia

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Background: Oxidative and metabolic stress responses signify cerebral cellular energy failure following perinatal hypoxic-ischemic (HI) injury. Creatine is a proposed prophylactic treatment to prevent HI brain injury due to its ability to buffer cellular ATP levels thereby preserving cellular energy supply during oxygen deprivation.

Aims/Hypothesis: We hypothesised that creatine would ameliorate metabolic collapse under HI conditions, and therefore reduce reactive oxygen species (ROS) production and HI-induced neuropathology.

Methods: Fetal sheep (118 days gestation [dGA]) were implanted with brain microdialysis probes, brachial arterial and venous catheters and an umbilical cord cuff. Creatine (6mg/kg/h) or saline (9mg/kg/h) was continuously infused from 122 dGA. At 131 dGA a 10-minute umbilical cord occlusion (UCO) was induced (n=8 creatine, n=7 saline) or sham occlusion (n=7 creatine, n=6 saline). Microdialysis efflux (2 µL/min) was collected in 1 h epochs and analysed for hydroxyl radicals (•OH) by terephthalic acid trapping and fluorescent HPLC and, concentrations of cellular metabolites by GC-MS. Data are presented as mean ± SEM. Overall change with time after UCO was assessed as area under the curve (AUC). Student's t-test, $p<0.05$ was considered significant.

Results: Creatine pre-treatment tended to lower •OH production post-UCO (26.31 ± 1.175 µM.h) compared to saline-infused fetuses (29.81 ± 1.556 µM.h) ($p=0.073$, AUC); and significantly reduced extracellular glycerol levels 14-72 h post-UCO (58.08 ± 7.79 vs 139.70 ± 29.6 µM.h saline-infused fetuses; $p=0.009$, AUC). Creatine treatment reduced basal extracellular cerebral pyruvate but did not prevent the increase in extracellular lactate concentration following UCO ($p>0.05$, AUC).

Conclusions: Creatine pre-treatment tended to reduce •OH and lowered glycerol cerebral extracellular accumulation during HI-mediated secondary energy failure. Analysis of tissue is underway to assess the impact of these findings on gene expression using RT-qPCR and neuropathology by immunohistochemistry.

Reducing inflammation in the growth restricted newborn to improve brain outcomes

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Background: The fetal brain is particularly vulnerable to intrauterine growth restriction (IUGR) and adverse outcomes in these infants range from behavioural and learning disorders through to cerebral palsy. Unfortunately, no treatment exists to protect the IUGR newborn brain and therefore reduce these life-long disabilities. Recent evidence suggests sustained inflammation in the IUGR brain following birth is associated with brain impairment in the IUGR newborn.

Hypothesis: We hypothesise that modulating the inflammatory response in the IUGR newborn will improve brain outcomes. We explored whether two treatments (ibuprofen or placental stem cells) that target inflammation could reduce brain impairment in the IUGR newborn piglet.

Methods: We used a preclinical piglet model of growth restriction where IUGR occurs spontaneously. Newborn IUGR (<10th centile) and normally grown piglets were collected on first day of life (P1). For the treatment groups:

Placental stem cells - Fetal mesenchymal stem cells (MSCs) and fetal endothelial colony forming cells (ECFCs) were recovered from healthy human term placentas through cell sorting and expanded in culture. 1mL placental stem cells (1 million MSCs and 1 million ECFCs) were administered i.v. to piglets on P1. Oral ibuprofen was administered to the piglets in piglet formula at each morning feed on P1 (20mg/kg/day) and on P2 and P3 (10mg/kg/day). Brain outcomes were examined on P4 in all treatment groups using immunohistochemistry and RNA analysis.

Results: IUGR brains demonstrated a proinflammatory state based on glial morphology (astrocytes and microglia) using immunohistochemistry, and an increase in a number of proinflammatory markers using qPCR.

Quantifying the longitudinal effects of periodic breathing in preterm infants

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Background: More than 10% of infants are born preterm (before 37 weeks gestational age (GA)) annually and the numbers are increasing. Cardio-respiratory control is immature in these infants and is often manifest as periodic breathing which, because the apnoeas are short, has been considered benign.

Aims/Hypothesis: To assess the frequency and severity of periodic breathing in preterm infants while in the nursery and after discharge, using physiological recordings of sleep and breathing. We hypothesised that periodic breathing frequency and severity would be associated with age and physiological changes**.**

Methods: 8 healthy preterm infants (5 males, 3 females) born between 29-33 weeks GA were studied with daytime polysomnography at 31- 36 weeks GA (n=8), 36-39 weeks GA (n=8), 3 months corrected age (CA) (n=7) and 6 months CA (n=8). Heart rate (HR), peripheral arterial oxygen saturation (SpO_2) and cerebral tissue oxygenation index (TOI) were recorded during active and quiet sleep. Periodic breathing episodes were defined as ≥ 3 sequential apnoeas lasting for ≥ 3 s with ≤ 20 s of normal breathing in between each apnoea.

Results: All infants exhibited at least one episode of periodic breathing at 31-36 weeks, 7/8 (87.5%) at 36-39 weeks, 6/7 (85.7%) at 3 months CA and 3/7 (42.9%) at 6 months CA. Time spent in periodic breathing and the associated physiological changes were not different between sleep states, so data were combined. Time spent in periodic breathing decreased significantly with postnatal age, but was variable between babies. Falls in HR and TOI related to apnoea events averaged -13 ± 3% and -4 ± 2% respectively at Study 1; -13 ± 3% and -5 ± 1% at Study 2; -14 ± 3% and -3 ± 1% at Study 3 and - 21 ± 7% and -2 ± 0% at Study 4.

Conclusions: Periodic breathing was very common in preterm infants and decreased in duration with increasing post-natal age. Falls in HR, SpO_2 and TOI persisted across the first 7 months of life.

Prenatal alcohol exposure results in sex-specific effects on placental glucocorticoid receptor and gene expression

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Background: Alcohol exposure during pregnancy results in elevated vulnerability to intrauterine growth restriction, preterm birth, miscarriage, and stillbirth in a sex-specific manner. Approximately 37% of full-term pregnancies are unplanned, with pregnancy recognition occurring around 6 weeks of gestation. Consequently, a large portion of women may be consuming alcohol around conception, during implantation, and throughout early development. While many of the detrimental effects of prenatal alcohol exposure on the fetus are thought to be mediated through placental dysfunction, the exact mechanisms remain unknown.

Aims/Hypothesis: We aimed to determine the effect of periconceptional alcohol exposure on glucocorticoid receptor expression and downstream gene signalling pathways. It was hypothesised alcohol exposure would contribute to a pro-inflammatory environment, with placentae from female offspring showing greater adaptation.

Methods: Participants (n=113) were recruited prospectively during early pregnancy between May 2009 and May 2012. Only singleton pregnancies were included. Amount and type of alcohol consumed was obtained by a questionnaire at 18 weeks of gestation. The level of drinking was separated into none (0 g/day), low (< 10g/day), moderate (10-100 g/day), and heavy (>100 g/day). Perinatal outcomes were collected at delivery, including placental weight, sex, and measures of growth. Placentae were collected for RNA and protein extraction. Glucocorticoid receptor (GR) isoform expression and downstream gene signalling pathways were quantified.

Results: Heavy alcohol exposure resulted in a cytoplasm specific increase in GRαC (P=0.07) and decrease in GRαD1 (P<0.05) expression in the placentae of female offspring only. There was also a trend for downregulated IL6R (P=0.06) and upregulated POU2F2 (P<0.01) gene expression in female and male placentae, respectively. Both male and female offspring born to heavy drinkers tended to be lighter at birth (90.9% born below 60th percentile, P=0.096).

Conclusions: These data suggest that heavy alcohol consumption alters placental GR-mediated inflammatory pathways, in a sex-specific manner, which may contribute to adverse birth outcomes.