Fetal & Neonatal Workshop of Australia and New Zealand
21st Annual Meeting

Royal Women's Hospital
Melbourne
March 31-April 1, 2007

2007 Organising Committee:

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Saturday March 31st

10:00 Registration / morning tea

Session 1 Brain Development

A1	11:00	**Ms Bobbi Slattery**	Effects of global birth asphyxia on hippocampal LTP in neonatal spiny mouse
A2	11:15	**Mr Tom Harris**	Does cortisol promote better maintenance of cardiovascular function and reduced neural injury following acute global hypoxia?
A3	11:30	**Ms Theodora Alexiou**	Angiotensin II induced cerebral vascular dysfunction in newborn lambs
A4	11:45	**Dr Flora Wong**	Cerebral intravascular oxygenation and autoregulation assessed by NIRS in the newborn lamb
A5	12:00	**Mr Aaron Smith**	Measurement of neurosteroids in the fetal and neonatal guinea pig brain using gas chromatography - mass spectrometry
A6	12:15	**Ms Olivia Wynne**	Impact of postnatal stress on neuroendocrine development and function in adulthood in the mouse
	12:30		General discussion

12:45 Lunch

Session 2 Renal / Cardiovascular

A7	1:45	**Dr Karen Gibson**	The renin response to haemorrhage is blunted in fetuses of subtotally nephrectomised ewes
A8	2:00	**Dr Alison Kent**	Renal injury following postnatal administration of indomethacin, ibuprofen and gentamicin in a neonatal rat model
A9	2:15	**Ms Stephanie R Yiallourou**	Dynamic changes of blood pressure during sleep in early infancy
A10	2:30	**Dr Amanda Brandon**	Programming of glomerular hypertrophy by maternal subtotal nephrectomy
A11	2:45	**Ms Reetu Singh**	Altered cardiovascular and renal function in male lambs following fetal unilateral nephrectomy
A12	3:00	**Mr Stephen Gray**	The effects of alcohol exposure on the developing rat kidney
	3:15		General discussion

3.30 Afternoon Tea

Session 3 Lung/Preterm Birth

A13	4:00	**Ms Beth Allison**	Changes in pulmonary blood flow during in-utero ventilation
A14	4:15	**Ms Megan O'Reilly**	Influence of intrauterine growth restriction on airway structure and alveolar attachments in adult sheep: a morphometric analysis
A15	4:30	**Dr Michelle Loeliger**	Premature birth: consequence for the developing visual system
A16	4:45	**Ms Caitlin Filby**	Pulmonary capillary embolisation disrupts alveolarisation – but does it cause hypoxia?
A17	5:00	**Dr Kelly Crossley**	Does caffeine affect pulmonary and ductus arteriosus blood flow and renal function in ventilated preterm lambs?
A18	5:15	**Dr David Todd**	Methods of weaning cpap in infants <30 weeks gestational age: a multicentre randomised controlled
	5:30		General discussion

5:45 Drinks

Sunday April 1st

8.30 Coffee

Session 4 Growth / Stress

A19	9:00	**Ms Michelle Bertucci**	What is the stimulus for elevated activin A in IUGR pregnancies?
A20	9:15	**Dr Anne Jaquiery**	Regulation of postnatal growth is altered by maternal periconceptional undernutrition
A21	9:30	**Mr Kyungjoon Lim**	Effect of intrauterine growth restriction on the number of cardiomyocytes in the rat heart at four weeks of age
A22	9:45	**Dr Michael Stark**	Placental 11 B hydroxysteroid dehydrogenase 2 and regulation of fetal adrenal function: the influence of exogenous gluco-corticoids and fetal sex in normal and growth restricted infants
A23	10:00	**Ms Kelly Kenna**	Effects of ethanol exposure during late gestation on physiological status and growth in fetal sheep
A24	10:15	**Ms Zoe Ireland**	Dietary creatine in the pregnant spiny mouse (*acomys cahirinus*) reaches the fetus and improves survival following birth asphyxia
	10:30		General discussion

10:45 Morning tea

Session 5 Placenta / behaviour

A25	11:15	**Dr Leo Leader**	Does antenatal maternal stress and anxiety influence fetal behaviour and infant development?
A26	11:30	**Ms Heidi Richardson**	Maturational changes in infant arousal processes during the first six months of life
A27	11:45	**Ms Nicole Smith**	Cardiovascular control differs in sleep in infants with a history of apnoea of prematurity.
A28	12:00	**Ms Wee-Ching Kong**	Micro-RNAs are differentially expressed between mid and late gestation in the mouse placenta.
A29	12:15	**Assoc Prof Claire Roberts**	IIGF-II synergises with low oxygen to promote placental trophoblast invasion
A30	12:30	**Ms Amanda Sferruzzi-Perri**	Acute effects of maternal IGF supplementation on placental gene expression, transport and nutrient partitioning in the guinea pig
	12:45		General discussion

1:00 Lunch

Session 6 Inflammation

A31	2:00	**Ms Andrea Lee**	The effects of intra-amniotic ureaplasma injection on gamma-delta T cells from the thymus and spleen of fetal sheep
A32	2:15	**Mr Adam Walker**	Impact of neonatal endotoxin exposure on later life anxiety-like behaviour in rodents
A33	2:30	**Ms Burcu Saglam**	Anti-inflammatory effects of sulfasalazine in an ovine model of *in utero* infection: a preliminary investigation
A34	2:45	**Ms Nadia Hale**	Erythropoietin as a potential therapeutic agent in the LPS-model of fetal brain injury: effects on the placenta and liver
	3:00		General discussion
	3:15		Presentation of Student Prizes

6:00 PSANZ Reception

EFFECTS OF GLOBAL BIRTH ASPHYXIA ON HIPPOCAMPAL LTP IN NEONATAL SPINY MOUSE

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Background and Aim: Long-term potentiation (LTP) is the long-lasting strengthening of the synaptic connection between two nerve cells, and is thought to be the physiological basis of memory and learning. In the hippocampus, changes in the strength and persistence of LTP are often studied at the synapses between Schaffer collateral axons and CA1/CA3 pyramidal neurons and reflect fundamental changes ('plasticity'). In this project we examined the effect of brief asphyxia at birth on the development of hippocampal LTP in neonatal Spiny Mouse.

Methods: The Spiny Mouse (*Acomys cahirinus*; SpM*)* is a precocial murid species particularly suitable for use in perinatal brain injury models since it displays a large number of spontaneous motor activities/reflexes that can be readily tested from birth and has an extended gestation (38-40 days) compared with conventional mice (21 days). The protocol for the induction of global birth asphyxia involves removing and submersing the intact uterus in a warm (37^{o}C) bath of saline on day 37 of gestation, and after 7-8 mins expelling and resuscitating the pups before cross fostering them to nursing dams.

Hippocampal slices (300 µm thick) were prepared from 6 and 15 day old pups obtained after normal vaginal delivery, caesarean section with no *in utero* asphyxia, or after 7-8 mins of global asphyxia. Extracellular electrophysiological recordings were made from the Schaffer Collateral Pathway, and LTP was induced by high frequency stimulation (HFS; 100Hz for 1sec x 4, 20sec interval). LTP was quantified by determining the maximal level of potentiation [MAX], and the degree of potentiation persisting at 2 h after initial induction [Persist.].

Results: LTP was similar in vaginally delivered and caesarean section (no asphyxia) animals, and was greater at 6 days of age (MAX = 280 ± 50%, Persist. = 150 ± 40%) than at 15 days (MAX = 170 ± 25%, Persist. = 145 ± 21%), and 40 days of age (MAX = 145 ± 34%, Persist. = 90 ± 31%). However, at 6 days after birth asphyxia LTP could not be induced in 4/5 pups, and in 1 pup LTP was transiently expressed but which decayed within 30 min to below control values. At 15 days of age, the maximal LTP was similar for all groups, but persistence (relative LTP amplitude at 2 h) was significantly less (Persist. =110%, n=2) in the asphyxiated pups compared with both the vaginally and caesarean delivered pups.

Conclusions: The structural and functional effects of asphyxia on cells in the neonatal hippocampus are not yet known, and may include death of neuronal and glial elements, or dysfunction leading to changes in glutamate release and re-uptake. Changes to neurons that result in alteration of LTP include not only neuronal loss, but also changes in dendritic arborisation and synaptic density. These possible structural changes, and change in expression of intracellular proteins involved with alterations of synaptic transmission, are currently being considered to explain the results shown above.

DOES CORTISOL PROMOTE BETTER MAINTENANCE OF CARDIOVASCULAR FUNCTION AND REDUCED NEURAL INJURY FOLLOWING ACUTE GLOBAL HYPOXIA?

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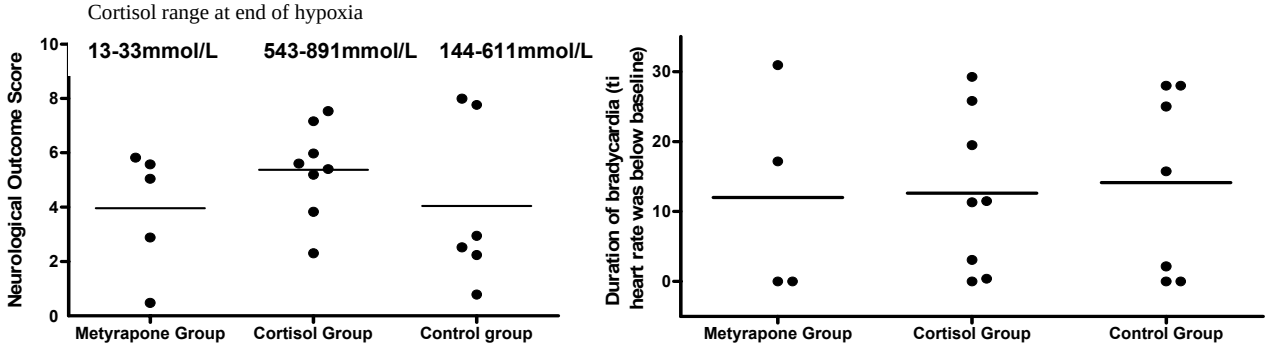
Background: Perinatal asphyxia is a significant contributor to neonatal brain injury. Our previous data has demonstrated that serum cortisol concentration is highly variable during hypoxia (240±90.9 nmol/L, mean ± SD), with higher cortisol levels at the end of hypoxia associated with better maintenance of cardiovascular function and less brain injury.

Aim: To identify if cortisol is the causative factor responsible for improved cardiovascular performance and reduced severity of brain injury following hypoxia.

Methods: Newborn piglets (n=19) were anaesthetized and ventilated. Arterial blood pressure, heart rate and EEG were monitored continuously. Arterial blood gases and glucose levels were measured intermittently. Blood samples were taken for the analysis of cortisol before hypoxia, every 15 minutes during hypoxia, and one, three and six hrs post hypoxia. Animals were separated into four groups. In one group of animals (n=5) metyrapone was injected (250mg/kg) 3hr before hypoxia, to inhibit cortisol production. In another group (n=8) cortisol was infused (200ug/kg/hr) during hypoxia to increase blood cortisol concentration to above 500nmol/L during hypoxia. There were two control groups (n=8) where a vehicle (cyclodextrin for cortisol or tartaric acid for metyrapone) was administered under the same conditions as the treatment groups. Hypoxic was induced in by reducing the $Fi0_2$ to 10% and reducing ventilation rate from 30 to 10 BPM for 45 min. Neurological outcome score was assigned using EEG and cerebral impedance data at 6hrs post hypoxia, where 0=bad outcome and 10=good outcome. ANOVA ($P<0.05$) was used to compare the serum cortisol levels, cardiac function and neurological outcome between the four groups.

Results**:** There was a statistical difference in the cortisol levels at the end of hypoxia between the metyrapone and cortisol group ($P = 0.001$). Baseline biochemical and physiological parameters did not correlate with neurological outcome. There was no difference between the two control groups so the data was combined. There was no difference between any of the groups in the neurological outcome score ($P = 0.79$), duration of hypotension ($P = 0.94$) or bradycardia ($P = 0.84$) during hypoxia.



Conclusion**:** This data indicates that there still is significant variability in neurological outcome regardless of the level of cortisol during hypoxia. When the cortisol level is manipulated to achieve levels consistent with good or bad outcome as previously reported, we observed neither improved outcome with high cortisol or worsening of outcome with low cortisol. This study demonstrated that cortisol is not directly responsible for better maintenance of cardiovascular function during hypoxia or reduced severity of brain injury following hypoxia.

ANGIOTENSIN II INDUCED CEREBRAL VASCULAR DYSFUNCTION IN NEWBORN LAMBS

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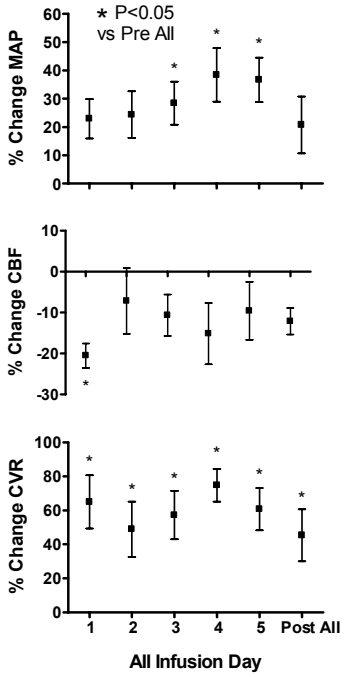
Background**:** Chronic angiotensin II (AII) treatment has been shown to increase the production of superoxide (O^{2-}), result in oxidative stress and lead to impaired endothelial-dependent vasodilatation of cerebral and thoracic arteries[1,2]. Endothelium derived vascular mediators, such as nitric oxide (NO), are key regulators of cerebral blood flow (CBF). However impaired endothelial function can alter NO availability and impair vascular compliance. During the perinatal period, oxidative stress and vascular dysfunction may arise in conditions such as inflammation, ischemia-reperfusion injury, and repeated apnea-hypoxaemia events in sleep. The objective of this study was to produce the first newborn animal model of AII induced vascular dysfunction for the investigation of such conditions.

Aims**:** The aim of this study was to induce cerebral vascular dysfunction in the newborn lamb by intravenous AII infusion.

Methods**:** AII was infused intravenously in lambs (n=7, age 9-15 days) for five days (Alzet Minipump Model 2ML1, 0.66mg/kg/day). Cerebral vascular function was assessed by measuring mean arterial pressure (MAP), intracranial pressure (ICP) and cerebral blood flow (CBF: measured via Transonic™ flow probe over superior sagittal sinus). Vascular resistance (CVR) was calculated as (MAP – ICP)/CBF.



Results**:** Compared to pre-AII infusion, MAP increased with AII treatment, reaching 38.4 ± 9.5 % above the control level on day four and 36.7 ± 7.9 % above the control level on day five of infusion ($P<0.05$). CBF was reduced on day one of AII infusion (-20.6 ± 3.0 %, $P<0.05$) but rose on subsequent days with sustained MAP increase. CVR was increased on day one of infusion (+65.0 ± 15.6 %) and remained elevated throughout the AII treatment period ($P<0.05$).

Conclusions**:** Chronic AII infusion led to impaired cerebral vascular function as indicated by the sustained increase in CVR and reduction in CBF after AII infusion. The impaired vascular function may be a result of endothelial dysfunction caused by an AII-induced excess of O^{2-} resulting in oxidative stress and altered vascular relaxation. This novel model of cerebral vascular dysfunction provides a means to study the perinatal regulation of CBF in conditions associated with impaired endothelial function.

References:

1. Rajagopalan, S., Kurz, S. et al. (1996). *The Journal of Clinical Investigation, 97(8):* 1916-1922.
2. Didion, S. P., Faraci, F. M. et al. (2003). *Stroke, 34:* 2038-2042.

Supported by the NHMRC of Australia

CEREBRAL INTRAVASCULAR OXYGENATION AND AUTOREGULATION ASSESSED BY NIRS IN THE NEWBORN LAMB

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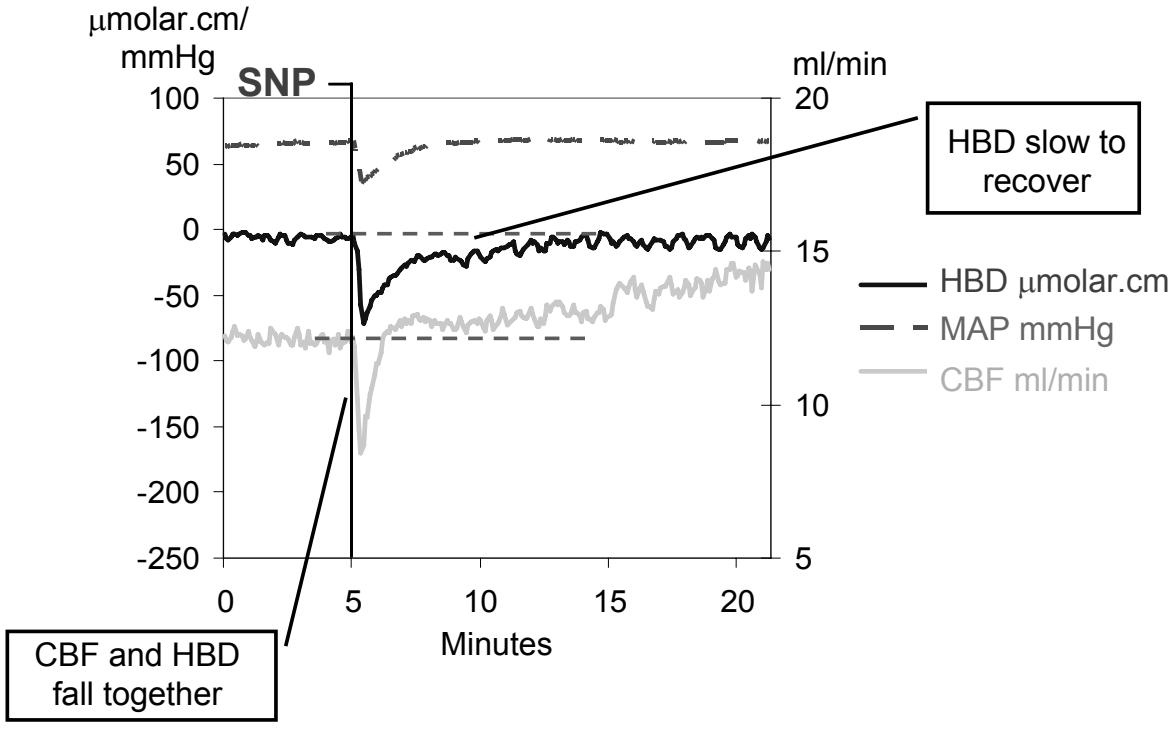
Aims: Near Infrared Spectroscopy (NIRS) measures changes in cerebral oxygenation and haemodynamics. Using continuous measurement of cerebral intravascular oxygenation (HBD) which is the difference between signals for oxyhaemoglobin (HBO) and deoxyhaemoglobin (HB), i.e. HBD=HBO-HB, we aimed to evaluate cerebral haemodynamic and oxidative changes during induced fluctuations in mean arterial blood pressure (MAP).

Methods: In ten newborn lambs (1-8 d of age) HBD was measured continuously with NIRS (Hamamatsu NIRO-500). CBF was measured with ultrasonic flow probe (TransonicTM) implanted around the superior sagittal sinus. MAP was recorded via an intra-arterial catheter and pressure transducer. MAP fluctuations were induced with intravenous sodium nitroprusside (SNP, 10 μg/kg)

Results: With rapid decreases of MAP, both CBF and HBD fell in close correspondence to each other. With restoration of CBF (autoregulation), HBD showed obvious delayed recovery (fig 1).

Conclusions: The slow recovery of HBD when CBF and MAP are restored indicates an increase in cerebral metabolic rate for oxygen following a short period of hypotension and reduced cerebral perfusion. Further studies are required to clarify the underlying mechanism.

Figure 1



MEASUREMENT OF NEUROSTEROIDS IN THE FETAL AND NEONATAL GUINEA PIG BRAIN USING GAS CHROMATOGRAPHY - MASS SPECTROMETRY.

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Background: Neurosteroids, such as pregnanolone (PREG) play a major role in the regulation of central nervous system (CNS) activity. These steroids are potent $GABA_A$ receptor agonists and play a role in reducing neuronal excitotoxicity through the GABAergic inhibition of CNS excitability. Pregnanolones have been shown to be present in the fetal brain at high levels and may be involved in protecting the fetus from brain injury during compromised pregnancies. However, the relative importance of potent neuromodulatory steroids including PREG and TH-DOC in the fetal brain and neuroprotection is not yet known.

Aim: The aim of this study was to validate Gas Chromatography-Mass Spectrometry methodology (GC/MS) for the measurement of the potent neurosteroids, PREG and TH-DOC in guinea pig brain tissue.

Methods: Steroids were measured in brain homogenates following C18 solid phase extraction. Samples were derivatised prior to GC/MS analysis in an optimised reaction using the silylation reagent BSTFA + TMCS (99:1). Derivatisation involved adding a volume of BSTFA + TMCS to dried sample extract residues and heating at 100°C for 1hr. The reactants were then dried and resuspended in chloroform prior to injecting into the GC/MS system. Steroid analysis was performed using a Quadrupole GC/MS system with electron-impact ionisation. GC separation was performed using a SGE Australia BPX-5, 30m x 0.25mm column with a 0.25um film thickness. MS analysis was performed using selective ion monitoring (SIM) mode analysis with the following program retention time and ion mass detection settings: PREG *m/z* = 300, 375 RT = 48.3 min; Alfaxalone *m/z* = 404, 260 RT = 51.5 min (internal standard); TH-DOC *m/z* = 257, 161 RT = 54.8 min; and DOC *m/z* = 387, 299 RT = 58.9 min. Concentration measurements were performed using the first pair of effective ion masses (*m/z*) with a second set used to improve compound identification. Steroid standards were used to calibrate the system and standard curve generation which were created through analysis of serial dilutions. All samples were analysed with a known concentration of internal standard added prior to derivatisation.

Results: The concentrations of PREG and TH-DOC were successfully measured using GC/MS methodology in maternal, fetal and neonatal brain samples. PREG concentrations were higher in fetal compared to neonatal guinea pig samples (fetal 220.6 $\pm$ 5.9 pmol/g and neonatal 136.8 $\pm$ 11 pmol/g, $P<0.05$, n=6) whereas there was no difference between fetal and neonatal brain TH-DOC levels (fetal 3.5 $\pm$ 0.1 nmol/g and neonatal brain 2.6 $\pm$ 0.13 nmol/g). Maternal brain PREG levels were generally lower than those in the fetus (80.6 pmol/g).

Conclusions: A GC/MS method has been developed with sufficient sensitivity to measure a range of levels of important neurosteroids in the fetal guinea pig brain using SIM mode analysis. The levels of PREG were higher in the fetal guinea pig brain compared to the neonate which is consistent with findings in other species. In contrast, similar TH-DOC levels were observed in both the fetal and neonatal brain, and levels were of higher concentrations compared to those for PREG. These findings suggest TH-DOC may have a major neuroprotective role in the fetal and neonatal brain particularly after birth.

IMPACT OF POSTNATAL STRESS ON NEUROENDOCRINE DEVELOPMENT AND FUNCTION IN ADULTHOOD IN THE MOUSE

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2. Mothers and Babies Research Centre, Hunter Medical Research Institute, Newcastle, NSW, Australia.
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Background: Prenatal and neonatal infection has been shown to alter glucocorticoid receptor function and the adult stress response in a number of animal models. However no studies have examined the paradigm in the mouse. This project focuses on the impact of early life exposure to immune activation on alterations in the hippocampus to the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR) at the gene, RNA and protein level; and the outcomes of such alterations on the adult stress response. Functioning will be determined by measuring GR, MR and corticotrophin releasing hormone (CRH) in the brain and measuring circulating corticosterone (CORT), corticosterone binding globulin (CBG) in the blood. An important aspect of this study is the development of a mouse model within the laboratory.

Aims: We are aiming to show that the early life programming we have shown in the rat is also present in the mouse, and wish to characterise the mouse endocrine response to neonatal infection and adult stress in the same way we have done in the rat. Given the mouse model's wide spread use of the mouse model in scientific research the ability to use this model in ongoing studies opens up the possibility of a variety of genomic manipulation methods not available in the rat.

Methods: At birth neonates were exposed to Chlamydia Muridarum (intranasally, 400 ifu). Control animals received no treatment. At nine weeks old animals were euthanized, brains removed and stored in RNAlater. The hippocampus was dissected and RNA, DNA and protein was extracted with the aim of measuring GR and MR mRNA abundance and methylation of DNA using real-time PCR as well as protein levels using western blot.

Results: A two-way ANOVA (sex x treatment condition) revealed a significant ($p < .05$) interaction between sex and treatment condition on abundance of MR abundance. Post hoc Bonferroni *t'* tests revealed that males in the neonatal infected group had a significant decrease in MR abundance compared to the male control group ($\alpha = 0.1$), while the female infected group showed a significant increase in MR abundance compared to female controls. A two-way ANOVA (sex x treatment condition) revealed no significant differences for GR abundance. Post hoc Bonferrroni *t'* tests revealed in the males, the neonatal infection group had significantly decreased GR abundance when compared to the control group ($\alpha = 0.1$). And females showed a significant increase in GR abundance when comparing the infected group to controls ($\alpha = 0.1$).

Conclusions: The current study suggests that changes to the stress response in the mouse may be mediated by alterations to GR and MR at the level of mRNA and that such alterations are sexually differentiated. Future studies aim to assess the levels of MR, GR and CRH at the level of protein. As well as measuring CRH and its receptors mRNA expression and the levels of methylation for GR, MR and CRH receptors. The use of the mouse model provides many scientific benefits. Many genes are highly conserved between mouse and human. Specific genes can be easily found in the mouse sequence and it is possible to experimentally test the function of the genes through transgenic models. The benefits of establishing the mouse model in this laboratory extend far beyond the current study.

Notes

THE RENIN RESPONSE TO HAEMORRHAGE IS BLUNTED IN FETUSES OF SUBTOTALLY NEPHRECTOMISED EWES

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Background and Aim: Fetal sheep carried by ewes that underwent subtotal nephrectomy prior to mating (STNxF) tend to have reduced plasma renin levels[1]. To further characterize the renin angiotensin system in these fetuses, we measured the renin response to haemorrhage in 6 STNxF and 7 control fetuses (IntF).

Methods: At least 2 months prior to mating maternal renal insufficiency was induced by subtotal nephrectomy under general anaesthesia. One kidney was removed and the remaining kidney was partially infarcted by ligation of one or more branches of the renal artery. At 115-122 days the fetuses and ewes were chronically catheterized under general anaesthesia. At 128-129 days fetuses were haemorrhaged (20% of estimated blood volume was removed over 20 min). Blood samples for renin levels were collected before, at 10 min and 20 min during haemorrhage and then at 10 min and 30 min after haemorrhage. Renin was measured as the rate of generation of angiotensin I (ng/ml/h) in samples incubated with nephrectomized sheep plasma (a source of angiotensinogen) at pH 7.5 and 37°C. At 129-133 days fetal weight was measured at post mortem, so that the amount of blood removed during haemorrhage could be accurately related to fetal weight at the time of the experiment.

Results: Prior to haemorrhage (CON), plasma renin levels were lower in STNxF than IntF (2.8 ± 1.0 (S.E.M.) versus 11.1 ± 5.9 ng/ml/h, $P<0.05$). Fetal mean arterial pressure was similar in the two groups (STNxF 41.1 ± 1.5; IntF 41.9 ± 1.0 mmHg), but haematocrit was lower in STNxF (23.1 ± 1.8% versus 29.6 ± 1.1%, $P<0.01$). The amount of blood removed during haemorrhage was 2.6 ± 0.1% of body weight in STNxF and 3.2 ± 0.3% in IntF (n.s.). In response to haemorrhage, mean arterial pressure fell in both groups ($P<0.01$). In the second 10 min of haemorrhage fetal mean arterial pressure was 28.5 ± 4.7 mmHg in STNxF (n=4; $P<0.01$ compared to CON) and 34.5 ± 1.7 mmHg in IntF (n=5; $P<0.01$ compared to CON). There was no difference in the blood pressure response between the groups. By 45 min after haemorrhage, haematocrit in the STNxF was reduced to 18.4 ± 1.2% ($P<0.01$ compared to CON), but in IntF haematocrit was not significantly altered (26.9 ± 0.8%). Thus, the haematocrit response was different between the groups ($P<0.001$). Although in STNxF a change in plasma renin levels was detected by ANOVA (after log transformation of the data), no difference from control values was detected by Dunnett's test. By contrast in IntF there was a prompt rise in plasma renin levels which was significant by 10 min into haemorrhage (21.1 ± 9.5 ng/ml/h, $P<0.05$ compared to CON) and was sustained. This renin response was different between the groups ($P<0.05$).

Conclusions: This study indicates that STNxF not only have suppressed renin levels under control conditions, but their renin response to haemorrhage is greatly attenuated. Since STNxF did not have a reduced hypotensive response to haemorrhage, this blunted renin response is not explained by reduced stimulation of the renal baroreceptors. The mechanism for the blunted response remains to be determined.

[1] Gibson et al., Am J Physiol Regul Integr Comp Physiol 292:R1204-1211, 2007.

RENAL INJURY FOLLOWING POSTNATAL ADMINISTRATION OF INDOMETHACIN, IBUPROFEN AND GENTAMICIN IN A NEONATAL RAT MODEL

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[1]Department of Neonatology, [2]Department of Anatomical Pathology, The Canberra Hospital, PO Box 11 WODEN, ACT 2606, Australia [3]Nephrologist, [4]Neurosciences Research Unit, Australian National University, Canberra, ACT, Australia, [5]Australian National University Medical School, Canberra, ACT, Australia.

Background**:** Indomethacin and ibuprofen are both used to close the patent ductus arteriosus in the premature infant. Gentamicin is frequently being administered at the same time. The morphological effect of these drugs individually, and in combination, on the developing kidney is unknown.

Aim**:** To determine whether postnatal administration of indomethacin or ibuprofen results in renal injury using light and electron microscopy.

Methods**:** Sprague-Dawley rat pups were administered intraperitoneally indomethacin, ibuprofen, gentamicin, or a combination of indomethacin and ibuprofen with gentamicin from day 1 to 5 of life (equivalent to 24-30 weeks in human fetus). The rat pups were sacrificed at 14 days of age (equivalent to term). The right kidney was removed and examined by light and electron microscopy for changes in the tubules and glomeruli.

Results**:**

	Groups							
LM Findings	Control N=15	Indo 0.1mg N=7	Indo 0.2mg N=4	Ibu 5mg N=9	Ibu 10mg N=6	Gent 2.5mg N=11	Indo 0.1mg & Gent 2.5mg N=5	Ibu 5mg & Gent 2.5mg N=6
Prox tubular epithelium cytplasmic vacuolization and granularity	+	+	++	+	+	+	++	++
Intraluminal vacuoles	+	+	++	+	++	+	++ focally +++	++ focally +++
Distal tubule vacuoles	-	-	+	-	+	+	+	+
Interstitial lymphocytes	+	+	+	+	+	+	+	+
Interstitial oedema	-	++	+	++	++	+	++	++
EM Findings	N=7	N=2	N=3	N=3	N=2	N=2	N=4	N=3
Foot process effacement	0%	25%	40%	35%	35%	40%	80%	50%
BM protrusions and lucency	-	+	++	+	++	+	+++	+++
Mitochondrial changes	-	+	++	+	+	+	+	+
Microvilli loss	-	-	+	+	+	+	+	+

Conclusions**:** Indomethacin, ibuprofen and gentamicin result in significant tubular and glomerular injury, the most severe injury noted when either non-steroidal drug was used in combination with gentamicin. The use of these drugs, particularly in combination, at a time of glomerulogenesis may risk long-term renal injury.

DYNAMIC CHANGES OF BLOOD PRESSURE DURING SLEEP IN EARLY INFANCY

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Monash University, Victoria, Australia.

Background: Epidemiological studies have revealed that a peak exists for Sudden Infant Death Syndrome (SIDS) between 2-3 mo postnatal age (PNA). In addition, the prone sleeping position has been identified as a major risk factor for SIDS. It is thought that circulatory failure may be a factor in the fatal event for SIDS. However, there is a paucity of data on the development of blood pressure (BP) changes during sleep in infants.

Aims: Our aims were to determine the effects of PNA on the development of BP control during sleep in the prone and supine sleeping positions over the first 6 mo of life.

Methods: Polysomnography was performed on 20 term infants at 2-4 wks, 2-3 mo and 5-6 mo PNA. A photoplethysmographic cuff (Finometer^{TM}) was placed around the infant's wrist to measure BP continuously. Measurements were recorded during quiet sleep (QS) and active sleep (AS) in both the supine (S) and prone (P) positions. BP was recorded in 1 or 2 min epochs with a 2 min rest period between each measurement, and measurements were repeated 4 times in each sleep state and each sleep position for each infant. Data were analysed beat-beat for mean (MAP), systolic (SAP) and diastolic (DAP) arterial pressure and a mean value calculated for each infant for each sleep state and position. The effects of PNA, position and state were compared using a 3-way RMANOVA for pair-wise comparison with Holm Sidak post hoc analysis.

Results: There was a consistent trend for BP to be lower at 2-3 mo PNA, and this was particularly prominent in the prone sleeping position (Table). There was an overall significant interaction ($p<0.05$) between PNA on MAP, SAP and DAP, however after a multiple comparison procedure the differences were too marginal to isolate the conditions which differed.

	MAP			**SAP**			**DAP**		
	2-4 WKS	2-3 MO	5-6 MO	2-4 WKS	2-3 MO	5-6 MO	2-4 WKS	2-3 MO	5-6 MO
QS-S	74 ± 4	73 ± 3	79 ± 4	89 ± 4	88 ± 3	93 ± 4	67 ± 3	65 ± 3	72 ± 4
AS-S	83 ± 5	79 ± 4	85 ± 4	97 ± 5	94 ± 4	100 ± 3	75 ± 5	72 ± 4	78 ± 4
QS-P	74 ± 4	68 ± 2	77 ± 3	88 ± 5	82 ± 2	89 ± 3	67 ± 4	62 ± 2	71 ± 4
AS-P	81 ±5	76 ± 3	82 ± 3	95 ± 5	91 ± 3	95 ± 3	73 ±4	69 ± 3	76 ± 3

Conclusions**:** These are the first data to examine the effects of sleep position on BP longitudinally over the first 6 mo of life. The trend for BP to be lower at 2-3 mo PNA, particularly in the prone position suggests a physiological decrease in vasomotor tone, in cardiac output, or in regional perfusion occurs at this age. This is a newly recognised developmental feature which may play an important role in SIDS and deserves further study.

PROGRAMMING OF GLOMERULAR HYPERTROPHY BY MATERNAL SUBTOTAL NEPHRECTOMY

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Background and Aim**:** Offspring of uninephrectomised mice have an increased glomerular number and size[1]. However, mice complete nephrogenesis after birth so effects on the offspring's development could be modified by the postnatal environment. We have developed a model of maternal renal dysfunction, in sheep a species which, like humans, completes nephrogenesis before birth. To see if maternal renal dysfunction altered renal development and activity of the renin angiotensin system of their offspring, we studied the renal morphology and the gene expression of components of the intrarenal renin angiotensin system (RAS) of lambs (STNxL, n=8) whose mothers had undergone subtotal nephrectomy at least 2 months before mating and pregnancy. STNxL were compared with lambs carried by ewes with intact renal function (ConL, n=10).

Methods**:** Lambs were euthanased by an I.V. injection of pentobarbitone sodium (2g, Lethabarb, Virbac (Australia), NSW) at ~10 days of age after an experiment to measure renal function including urinary protein excretion. Both kidneys were weighed and photographed and the right kidney was bisected longitudinally and immersed in 4% paraformaldehyde in 0.5M phosphate buffer for later glomerular number and volume estimation by an unbiased stereological technique. Pieces of the left kidney were snap frozen for analysis of mRNA levels using real-time PCR for renin, angiotensinogen and the angiotensin receptors type 1 and 2 (AT_1R and AT_2R).

Results**:** Birth weights, weight at postmortem and kidney to body weight ratios were similar between the two groups as previously reported[2]. While glomerular number was very similar (STNxL 429,530 $\pm$ 27,471 (SEM) v ConL 423,520 $\pm$ 22,194 glomeruli), mean glomerular volume (STNxL 1.09 $\pm$ 0.04 $x10^{-3}$ v ConL 0.85 $\pm$ 0.04 mm^3, $p<0.001$) and total glomerular volume (STNxL 467 $\pm$ 36 v ConL 356 $\pm$ 21 mm^3, $p<0.01$) were larger in the STNxL. In STNxL there was a positive relationship between total glomerular volume and urinary protein excretion ($r^2=0.528$; $p<0.05$). This was not seen in ConL. There was no difference in protein excretion between STNxL and ConL. Renal mRNA levels for renin, angiotensinogen, AT_1R and AT_2R (relative to 18S) were similar in both groups.

Conclusions**:** The results of this study suggest that exposure to an altered intrauterine environment resulting from prior maternal subtotal nephrectomy programs for glomerular hypertrophy in the offspring. This difference in glomerular morphology was not accompanied by any alteration in expression of the intrarenal RAS at this age. The 30% increase in glomerular size and the presence of a relationship between glomerular size and urinary protein excretion suggests that offspring of pregnancies where maternal renal function is compromised may be predisposed at an early stage of life to age dependent deterioration in renal function.

References

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ALTERED CARDIOVASCULAR AND RENAL FUNCTION IN MALE LAMBS FOLLOWING FETAL UNILATERAL NEPHRECTOMY

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Background: There is accumulating evidence that being born with a lower number of nephrons is associated with an increased predisposition to cardiovascular disease. Children born with unilateral renal agenesis have impaired renal function and elevated blood pressure. However, removal or loss of a kidney in adult life (i.e, kidney donation) does not usually result in elevated blood pressure. This suggests that the events that alter kidney development resulting in a reduced nephron endowment at birth may increase ones predisposition to adult disease rather than a nephron deficit *per se*. We hypothesised that removal of a fetal kidney in the active period of nephrogenesis (100d gestation in sheep) would alter the entire cardiovascular system and result in elevated blood pressure in male lambs at 6 mths of age.

Aim: We aimed to determine the effect of fetal uninephrectomy (uni-x) in the active period of nephrogenesis on: (1) Mean arterial pressure (MAP); (2) cardiovascular parameters namely, cardiac output (CO), stroke volume (SV), total peripheral resistance (TPR), central venous pressure (CVP); (3) cardiovascular function and (4) renal function in 6 mth old male lambs.

Results: At 6 mths of age uni-x males had significantly elevated MAP (91 ± 3 vs 77 ± 3 mmHg; mean ± SD; n=5/ group, $P < 0.001$), CO (161 ± 26 vs 115 ± 4 ml/kg/min; $P < 0.01$), SV (1.8 ± 0.4 vs 1.2 ± 0.2 ml/kg/beat; $P < 0.05$) and CVP (6 vs 3 mmHg; $P < 0.001$) compared to sham animals. Plasma creatinine was also significantly elevated in uni-x animals at 6 mths of age (88 ± 2 vs 67 ± 5 µmol/l; $P < 0.05$). Water intake and urine output were not different between the groups but urinary sodium excretion was significantly reduced (51.1 ± 11.4 vs 151.1 ± 30.2 mmol/day; $P < 0.01$) and urinary albumin excretion was significantly elevated (135 ± 25 vs 0 µmol/day).

Conclusion**:** A reduced nephron complement as a result of uninephrectomy in the active period of nephrogenesis results in elevated blood pressure which appears to be driven by increased CO and SV. Uni-x animals also have a significantly reduced cardiac functional reserve (CO_{max}) in response to a cardiac challenge suggesting severe cardiac impairment at an early age. The elevation in serum creatinine suggests that these animals are at a higher risk of progressive renal disease. The urinary parameters suggest that the remnant kidney may have undergone “abnormal” remodelling resulting in altered tubular reabsorption and glomerular filtration.

THE EFFECTS OF ALCOHOL EXPOSURE ON THE DEVELOPING RAT KIDNEY

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Background: Consumption of large doses of alcohol during pregnancy is known to lead to neurological, craniofacial and cardiovascular malformations in offspring. However, little is known of the affects of alcohol exposure on the developing kidney. Our laboratory has recently shown that rat pups exposed to a moderate dose of ethanol (6%v/v) daily *in utero* have a reduced nephron endowment, and male offspring exposed to ethanol *in utero* have an elevated systolic blood pressure. However, no previous study has evaluated the effects of ethanol exposure in an acute setting on kidney development or subsequent nephron endowment and development of adult disease. Our hypothesis was that ethanol exposure to the developing rat kidney will result in smaller kidneys with fewer nephrons. These kidneys will have an impaired function in adulthood.

Aims: Adopting a rat *in vivo* and *in vitro* model, to evaluate the effects of ethanol exposure on kidney development and development of adult diseases.

Methods: *In vitro* – To assess if ethanol can directly affect ureteric branching morphogenesis, metanephric organ culture will be used. Whole metanephroi from time-mated Sprague-Dawley rats at embryonic day 13.5 (E13.5) will be randomly assigned to one of the four study models and then grown in different concentrations of ethanol. The ethanol concentration to be used for each of the four study models is culture media supplemented with either 0% (control), 0.06%, 0.13% or 0.2% ethanol. These concentrations of ethanol have been selected as they represent a blood alcohol concentration (BAC) of 0.00g/dL, 0.05g/dL, 0.10g/dL and 0.15g/dL respectively. The four study models are: Ethanol Metabolism Study (analysis of ethanol concentration within the ethanol-supplemented culture media), Cyclic Ethanol Exposure (ethanol-supplemented culture media administered every 24 hours), Acute Ethanol Exposure (one exposure to ethanol-supplemented culture media) and Chronic Ethanol Exposure (ethanol concentration maintained for 48 hours). At the end of the culture period the number of ureteric branch points and glomeruli in metanephroi will be determined. This is performed using immunostaining with monoclonal Anti-Pan Cytokeratin antibody for visualisation of the ureteric tree (branch points) and Rhodamine-Labelled Peanut Agglutinin lectin for visualisation of glomeruli.

Anticipated Outcomes: In E13.5 metanephroi cultured in physiological relevant concentrations of ethanol, there will be a reduction in the number of ureteric branch points and glomeruli.

Methods: *In Vivo* – Time-mated Sprague-Dawley rats will be administered by gavage 2g/kg of ethanol diluted in saline on E13.5 and E14.5. The anticipated BAC of this ethanol dose is approximately 0.11g/dL. Two control groups will be used (sham exposed and untreated). Offspring from the three treatment groups will be weighed every 3 days after birth and their head circumference and crown-rump length measured. Study cohorts will be culled at postnatal day (PN)30 and PN360. At PN30, 8 males and 8 females from the treatment, SHAM and control groups will be culled for stereological analysis, to determine total nephron number. At PN360, conscious mean arterial blood pressure will be measured by telemetry. Animals will be anaesthetized and a catheter inserted into the jugular vein and bladder for measurement of glomerular filtration rate (GFR) using inulin.

Anticipated Outcomes: Pups exposed to ethanol for a short period of time during gestation will be smaller at birth and remain smaller postnatally. They will have smaller kidneys with fewer nephrons. This will result in an elevation of blood pressure and a reduced GFR.

Notes

CHANGES IN PULMONARY BLOOD FLOW DURING *IN-UTERO* VENTILATION

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Background**:** Increased pulmonary blood flow (PBF) is an essential component of the transformation of the lung into a functional gas exchange organ at birth. Previous studies have established that the onset of ventilation is one of the precipitating factors regulating the increase in PBF normally seen at birth. Determining the effect of mechanical ventilation on PBF is important as low PBF can lead to ventilation perfusion mismatching. We have recently developed a model of *in utero* ventilation that allows the investigation of factors contributing to lung injury in very preterm infants. Given that the pulmonary vascular system is very immature at the time of ventilation we aimed to determine how this mode of ventilation in conjunction with the immaturity of the pulmonary system would affect PBF.

Objective: To measure the changes in pulmonary blood flow during *in utero* ventilation in very immature fetuses (110 days of gestation).

Methods**:** A tracheotomy was performed in fetal sheep at 105d GA (n=4; term ~147d) to insert an endotracheal tube which was connected to a neonatal ventilation circuit. A transonic flow probe was also placed around the left pulmonary artery. At 110d GA, lung liquid was drained and fetuses were ventilated for 12 hrs, throughout which, continuous measurements of pulmonary blood flow were made. Following the ventilation period the lung liquid was replaced and the fetus was allowed to develop in utero for a further 7 days.

Results**:** Pulmonary blood flow changes appeared to be quite variable between animals. One had a transient increase (as great as 2099.7% increase from control) in PBF following the initiation of ventilation (Fig 1.), whereas three other fetuses had variable responses to ventilation *in utero* (Fig 2.). Variability appeared to be greatest during the first three hours of the ventilation regime and towards the end of the 12 hour ventilation regime.

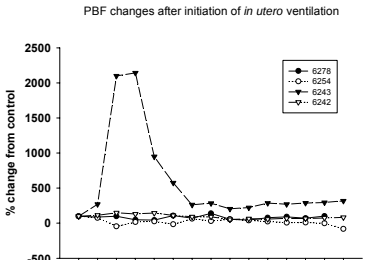

Figure 1. Changes in PBF during ventilation: all animals shown.

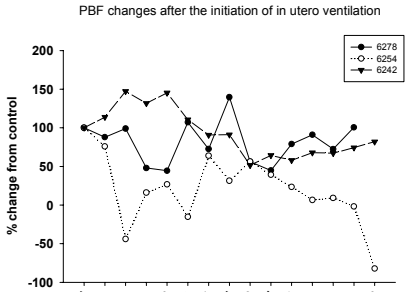

Figure 2. Changes in PBF during ventilation: outlier excluded

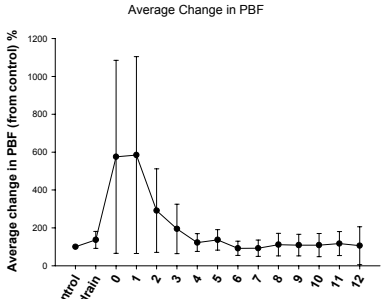

Figure 3. Average change in PBF from control.

Conclusions**:** Ventilation of fetal sheep *in utero* causes PBF changes which are very variable. The cause of this variability is unknown and is an area for future research.

INFLUENCE OF INTRAUTERINE GROWTH RESTRICTION ON AIRWAY STRUCTURE AND ALVEOLAR ATTACHMENTS IN ADULT SHEEP: A MORPHOMETRIC ANALYSIS

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Background: Intrauterine growth restriction (IUGR) and placental insufficiency are common causes of low birth weight. Epidemiological studies have shown associations between low birthweight and reduced lung function and an increased risk of death from respiratory causes in adults (Barker 1998); these studies suggest that airway development may be permanently altered by IUGR. In a recent study by Maritz et al (2004) it was shown that IUGR during late gestation in sheep leads to a reduction in alveolar number, observed at 8 weeks and 2 years after birth. A reduced alveolar number could reduce the tethering (ie external support) of bronchioles by alveoli, which could in turn impair airway function. However, there is little information about the long term effects of IUGR on airway structure and alveolar attachments.

Aim: The aim of the present study was to determine the effects of IUGR on the structure of the small pulmonary airways and alveolar attachments in the adult sheep.

Methods: IUGR was induced in pregnant sheep by umbilical-placental embolization, which was maintained from 120 days of gestation until the onset of labour at term (~ 147 days). Lambs were initially raised with their mothers and after weaning were kept as a flock until two years of age. They were then humanely killed and the right lung fixed at 20 cm H_20 via the trachea. Lung tissue from the middle and lower lobes was sectioned at 5μm and stained with Masson's trichrome. For each animal (8 control, 12 IUGR), 10 bronchioles which had been sectioned at right angles from each lobe were selected for analysis using Image Pro Plus. For each airway we analysed lumen area, basement membrane perimeter, and areas of epithelium, smooth muscle and collagen; the number of alveolar attachments was also analysed for each bronchiole.

Results: The bronchioles in IUGR lungs, compared with controls, did not have alterations in size or wall structure; lumen area, basement membrane perimeter, and adjusted areas of epithelium, collagen and smooth muscle did not differ significantly between groups. In IUGR lungs (both lobes combined), alveolar attachment number was ~10% less than that of controls: controls 23.9±0.5 vs IUGR 21.8±0.4 attachments / mm basement membrane.

Conclusions: Our data show that the airway walls of adult IUGR sheep were not different from those of controls. However, the number of alveolar attachments on bronchioles was reduced in adult IUGR sheep. As these small airways play a key role in tethering bronchioles, a reduced number of attachments could increase the risk of airway narrowing, thus impairing later lung function.

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PREMATURE BIRTH: CONSEQUENCE FOR THE DEVELOPING VISUAL SYSTEM

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Background and Aim: Survival of premature infants has greatly increased in recent years due to advances in prenatal and neonatal care, and in particular to the advent of postnatal ventilatory therapies. Presently however, little is known about the effect of these therapies on the developing retina. We have evaluated the influence of early nasal continuous positive airway pressure (EnCPAP) ventilation or delayed (Dn)CPAP, therapies which are routinely employed in neonatal intensive care units (NICU) in a primate model (baboon *papio sp*.) of premature birth. Prior to assessing structural alterations to the retina following premature delivery and postnatal ventilation it was firstly necessary to understand the normal maturational processes of the retina.

Methods: To characterize the growth of neural processes and cells comprising the retina gestational control animals were delivered at 125 (n=2), 140 (n=2) and 160dg (n=3) at the Southwest Foundation. In the experimental study baboons were prematurely delivered (PD) at 125 days of gestation (dg, term ~185dg) by caesarean section. Infants were ventilated for 28 days post-delivery with either: EnCPAP (n=6) or DnCPAP (n=5). Gestational controls (n=4) were delivered at 153dg. At the completion of the study period animals were euthanased with sodium pentobarbitone (130mg/kg i.v.) and retinae were embedded in Epon-Araldite for structural analysis.

Results: The developing retina has an immature appearance at 125dg with 3 discernible layers: a neuronal layer (NL); an inner plexiform layer (IPL); and a ganglion cell layer (GCL). At 125dg the outer (O)PL is a discontinuous thin zone. Occasionally intensely stained and slender cell bodies are observed migrating across the neuronal layer. Apoptotic and mitotic figures were observed but infrequently. At 140dg all 7 adult layers are evident due to the separation of the neuronal layer into an INL and an ONL separated by an OPL and photoreceptor outer and inner segments are just emerging. Cellular migration and mitotic figures are no longer observed; apoptotic bodies are observed infrequently. By 160dg all retinal layers are clearly visible and photoreceptor outer and inner segments are more fully developed. No overt structural alterations were observed in either PD group; retinal widths are currently being measured. Immunohistochemistry is also being carried out in these animals to assess astrocytes and their relationship with the vasculature and specific populations of retinal neurons including amacrine and horizontal cells.

Conclusion**:** The baboon retina is immature at 125dg, the time point at which animals are prematurely delivered. Premature delivery does not appear to cause major damage within the retina but could cause more subtle effects in specific cell groups and connectivity.

PULMONARY CAPILLARY EMBOLISATION DISRUPTS ALVEOLARISATION – BUT DOES IT CAUSE HYPOXIA?

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Background: Infants with chronic lung disease exhibit an arrest of alveolar development, with fewer larger alveoli and abnormally developed peri-alveolar capillaries. It has been suggested that signalling between the pulmonary endothelial cells and the myofibroblasts responsible for secondary septal crest formation is disrupted by ventilator-induced injury which prevents normal alveologenesis. We hypothesised that targeted disruption of the pulmonary vascular bed would arrest capillary development and, as a result, prevent the process of secondary septation and alveolarisation. We have since established the effect of left pulmonary arterial embolisation (LPAE), on fetal lung development. LPAE did not affect the amount of elastin deposited in the lung, however the type of deposition was abnormal, predominantly occurring in the parenchyma and around the base of alveoli. Septal crest density was significantly decreased and total tissue density was increased following LPAE. The proportion of lung tissue stained for collagen fibres was significantly increased following LPAE, despite a reduction in α-smooth muscle actin (αSMA; a marker of mature myofibroblasts). Hence, our data suggest that insults to the pulmonary capillary network during development, may lead to an arrest of alveolarisation in the developing lung. However the exact mechanisms by which this occurs remains unknown - does LPAE cause tissue hypoxia in the fetal lung? Is hypoxia the stimulus which causes altered secondary crest development or is it the loss of mesenchymal-epithelial interactions?

Aim: To determine the effect of pulmonary embolisation on biochemical markers of tissue hypoxia, pulmonary capillary development and secondary crest development.

Proposed Methods: Pregnant ewes will undergo surgery at ~105 days of gestational age (GA; term ~147d) to implant fetal vascular and tracheal catheters. At 110d GA, 5 injections of 1 million (in 1ml) 15μm microspheres will be injected into the left pulmonary artery every 10min. This will be repeated for 5 days (5d LPAE, n=5). The opposite side of the lung will be used as a control. On day 6 (115d GA), fetuses will receive a single injection of Hypoxyprobe-1 (100mg/kg) into the fetal jugular vein ~2hrs before post-mortem. Animals will be killed at 115d GA, fetal lungs were removed, weighed, divided into lobes and each lobe further divided into 3 areas. Each region will be weighed before small pieces of the tissue are snap frozen in liquid nitrogen and stored at -70°C. Small sections from each area of the lung will be used determine mRNA expression of markers of vascular development (platelet endothelial cell adhesion molecule, endogenous monocyte activating protein II, IL-8), alveolar development (αSMA and tropoelastin) and hypoxia (hypoxia inducible factor-1α) using quantitative real time PCR. Regions of hypoxia will be confirmed in similar areas using immuno-histochemistry to detect Hypoxyprobe-1. The remaining tissue for each area of the lung will be digested using a microsphere extraction protocol and the number of microspheres per region counted using a haemocytometer. Hence, the severity of embolisation (number of microspheres) will be compared to the expression of the various biochemical markers and regional hypoxia as detected by the Hypoxyprobe-1.

We expect that the results of this study will (i) provide evidence for the early biochemical/molecular changes that occur in fetal lung structure following LPAE, (ii) establish whether regions of hypoxia occur normally in the fetal lung and (iii) whether LPAE results in hypoxia of the fetal lung.

DOES CAFFEINE AFFECT PULMONARY AND DUCTUS ARTERIOSUS BLOOD FLOW AND RENAL FUNCTION IN VENTILATED PRETERM LAMBS?

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Background and Aim: Caffeine is commonly used in the clinical management of very preterm infants (<28 weeks of gestation), particularly in infants that have an immature breathing pattern. Caffeine is a respiratory stimulant that reduces the frequency of apneic events. A recent study has demonstrated that caffeine usage in very preterm infants is associated with a significant reduction in chronic lung disease (CLD) from 47% in the control group to 36% in those given caffeine and these infants had a reduced rate of patent ductus arteriosus (DA; Schmidt et al. 2006). CLD is an important outcome of these infants both because of its economic costs (more days on assisted ventilation) and its association with long term pulmonary and neurological problems. However, the mechanisms of the beneficial effects of caffeine in reducing CLD in infants are unknown. We hypothesise that caffeine increases urine output which aids in the clearance of liquid from the lungs and reduces the likelihood of pulmonary oedema in these preterm infants.

Methods: Catheterised fetal sheep, with transonic flow probes around the left pulmonary artery (PA) and DA were delivered by caesarian section at 125±3 days of gestation. Lambs were ventilated with a tidal volume of 5 mL/kg, rate 60/min, variable fraction of inspired oxygen (FiO_2) and 5 cm H_2O positive end-expiratory pressure for 3 hours. PBF and DA flow were digitally recorded before, during and after the onset of ventilation at birth. A bladder catheter was inserted soon after delivery. After a 30 minute stabilisation period, lambs were randomly given 40 mg/kg caffeine base i.v (n=2) or a saline infusion (control lambs; n=2) over 30 minutes. Lambs were then ventilated for 2 hours. Arterial blood samples (0.25ml) were regularly collected to monitor the pH, oxygen and carbon dioxide levels in the fetal blood. The lamb bladder was drained for urine and any urine output collected over a 30 minute period was pooled for analysis of flow rate, osmolality and electrolyte (sodium and potassium), creatinine and urea concentrations. Ventilation parameters, such as respiratory compliance, minute ventilation, peak inspiratory pressure and mean airway pressure were recorded every 10 minutes. Lung tissue was collected for histological analysis.

Results: Preliminary results indicate that there are no obvious differences between caffeine treated and control ventilated preterm lambs in PA or DA blood flow, ventilation parameters, urine output, renal function or arterial blood gas status.

Conclusion: Further studies are required to elucidate the beneficial effect of caffeine in reducing CLD.

Schmidt et al., 2006. Caffeine therapy for apnea of prematurity. N Engl J Med 354; 2112-2121.

METHODS OF WEANING CPAP IN INFANTS <30 WEEKS GESTATIONAL AGE: A MULTICENTRE RANDOMISED CONTROLLED TRIAL

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Background: Continuous positive airway pressure (CPAP) is now part of routine respiratory care for preterm infants either as a primary or secondary treatment following extubation. There are several methods of weaning the CPAP "off" these infants, but in general this is "ad hoc" and depends on previous experience and little scientific evidence!

Aim: Our primary aim is to determine the duration of support for each weaning method and our secondary aim is to study the influence of weaning CPAP on the incidence of chronic lung disease.

Methods: From March 2006 we have been recruiting infants <30 weeks gestation and randomly assigned then to 1 of 3 different methods of weaning CPAP. The 3 methods are:

1) The infant is taken "OFF" CPAP with the intent to remain "OFF" and remains in crib oxygen/air;
2) CPAP is weaned gradually by giving fixed increasing periods of time "OFF" and the infant remains in crib oxygen/air during their time "OFF" CPAP;
3) As in "2", CPAP is weaned gradually by giving increasing fixed periods of time "OFF", but are given oxygen/air via nasal cannula during their time "OFF" CPAP.

Results: From March 2006 to March 2007 we have recruited 43 patients. Their mean gestational age at birth was 26.9±1.4 weeks, and birthweight was 908.0±273.2 grams; and there was an even distribution between the 3 methods (table).

	Method 1 (n=16)	Method 2 (n=13)	Method 3 (n=14)
Gestation (weeks)	27.1±1.2	26.7±1.8	26.9±1.4
Birthweight (grams)	914.4±268.0	951.3±263.9	854.6±298.0
Gender (M:F)	8:8	6:7	9:5

Conclusion: To date we have not encountered any problems with either method and parents are willing to have their infants enrolled. We have been recruiting at Westmead NICU for one year and at Canberra NICU for 2 months. We invite comments and other units who wish to be part of this important trial (Perinatal Trials registration number ACTRN012606000155594).

Notes

WHAT IS THE STIMULUS FOR ELEVATED ACTIVIN A IN IUGR PREGNANCIES?

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Background**:** Intrauterine fetal growth restriction (IUGR) describes infants with a birth weight below the 10th percentile for gestational age, with a pathological restriction of fetal growth due to adverse genetic or environmental factors. The mechanisms underlying IUGR are complex and remain poorly understood. Activin A, a glycoprotein hormone, has been shown to be significantly elevated in both human and ovine pregnancies affected by IUGR. The role of activin A in such pregnancies remains to be elucidated and the stimulus for activin A production and increase in IUGR is not known, however previously in acute studies we have shown that increases in activin A are related to fetal oxygenation, pH, and prostaglandin E_2 (PGE_2) changes. Other studies have also shown that activin A is increased in response to acute LPS-induced inflammation.

Aim: The aim of this project is to look at factors which may contribute to the increased activin A in IUGR. We will use our established model of single umbilical artery ligation (SUAL) to induce IUGR in fetal sheep.

Methods**:** Surgery occurred at 109±1.0 days (term ~147 days) in four ewes carrying twins (n=4). This allowed for the implantation of catheters into the fetal femoral artery, amniotic fluid cavity, maternal jugular vein and the ligation of the uterine artery (SUAL) in one twin with the remaining twin subject to sham ligation. Plasma and amniotic fluid samples were taken across the experimental period of 7 days to measure fetal oxygenation and pH, activin A using ELISA and PGE_2 measured by RIA.

Results**:** SUAL fetuses displayed significant reductions ($p<0.05$) in body weight when compared to controls (1.71±0.08kg and 2.21±0.16kg), with significantly increased ($p<0.05$) brain to body weight ratio (19.43±1.0kg and 15.69±1.0kg). This demonstrates that SUAL induced asymmetric fetal growth restriction. Fetuses subjected to SUAL quickly became hypoxic, with a significant reduction ($p<0.05$) in oxygen saturation (sO_2) compared to controls (42.08±6.44% and 69.28±4.09%). pH values showed a significant change over time. Activin A in amniotic fluid was found to be significantly elevated ($p<0.05$) in SUAL fetuses by 80 hours compared with controls, with a significant increase ($p<0.05$) over time in this group. Amniotic fluid PGE_2 was found to be significantly increased ($p<0.05$) in SUAL fetuses at 23 hours post surgery (14.01±8.9ng/ml and 4.39±3.23ng/ml). A negative correlation was found between sO_2 and amniotic fluid activin A ($r=-0.333$ $p=0.00217$) and a positive correlation between amniotic fluid PGE_2 and activin A ($r=0.334$, $p=0.00176$).

Conclusions**:** SUAL induces asymmetric fetal IUGR, hypoxia, acidemia, increased PGE_2 and activin A, as seen in human IUGR. To date, we have found that the increased activin A in SUAL-induced IUGR is correlated with changes in fetal oxygenation, pH and PGE_2. We will continue to investigate these changes over time and to more closely examine the temporal relationship of these factors in response to SUAL.

REGULATION OF POSTNATAL GROWTH IS ALTERED BY MATERNAL PERICONCEPTIONAL UNDERNUTRITION

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Background: Periconceptional undernutrition has been shown to affect fetal physiology and growth trajectory, without affecting birth weight. Less is known about its effects on postnatal growth, which is determined primarily by nutrient intake and uptake, growth related hormones and genetic potential.

Aims: The aims of this experiment were firstly to assess the effects of periconceptional undernutrition on early postnatal growth, and secondly to explore the relationships between lamb growth, nutrient intake and growth-related hormones.

Methods: Singleton offspring of normally nourished (N, n=18) or periconceptionally undernourished (UN, n=11) ewes were weighed and measured at birth and weekly for the first 12 weeks of life. In UN ewes, the nutritional restriction was from 60 d before to 30 d after mating and was manipulated to result in a 10-15% reduction of body weight. All ewes were normally nourished from 30 d onwards. Plasma concentrations of metabolites (glucose, free fatty acids, urea and amino acids) and growth related hormones (IGF-1, insulin and cortisol) were measured at birth and 1, 6 and 12 weeks of age. Nutrient intake was assessed by D_2O dilution in the second week of life. Differences between nutritional groups were assessed by ANOVA with Fisher's post hoc test. Relationships between the growth and its regulating factors were assessed by multiple regression analysis. Significance was taken as $p<0.05$.

Results: There were no differences between nutritional groups in birth weight, lamb growth velocity in the first 12 weeks of life, or in plasma metabolite concentrations. Plasma IGF-1 concentrations were greater in lambs of UN than N ewes at birth (101±8 vs. 62±11 $ng.ml^{-1}$ $p<0.01$) and one week of age (185±10 vs. 144±23 $ng.ml^{-1}$ $p<0.05$) but not thereafter. There were no differences between nutritional groups in plasma concentrations of insulin or cortisol, nor in milk intake. In lambs of N ewes, there were positive associations between milk intake and growth velocity ($R^2=0.25$, $p=0.006$); between weight and plasma insulin concentration at 6 and 12 weeks (6 weeks $R^2=0.76$, $p=0.01$; 12 weeks $R^2=0.73$, $p=0.03$); and between plasma insulin and IGF-1 concentrations at birth and 6 weeks (birth $R^2=0.60$, $p=0.04$; 6 weeks $R^2=0.70$, $p=0.02$). None of these associations were significant in lambs of UN ewes. Plasma IGF-1 concentrations at 1, 6 and 12 weeks were positively associated with weight in lambs of ewes from both nutritional groups, but more strongly in those of N ewes (1 week, $R^2 = 0.29$, $p=0.02$; 6 weeks, $R^2 = 0.32$, $p=0.001$; 12 weeks, $R^2 = 0.43$, $p<0.001$) than in lambs of UN ewes (1 week, $R^2 = 0.11$, $p=0.12$; 6 weeks, $R^2 = 0.30$, $p=0.009$; 12 weeks, $R^2 = 0.17$, $p=0.05$).

Conclusions: The relationships between growth-regulating factors and postnatal growth are altered in lambs of periconceptionally undernourished ewes. This suggests that postnatal growth regulation can be altered by events that occur very early in intrauterine life.

EFFECT OF INTRAUTERINE GROWTH RESTRICTION ON THE NUMBER OF CARDIOMYOCYTES IN THE RAT HEART AT FOUR WEEKS OF AGE

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Background: During fetal development, myocardial growth is regulated by controlled proliferation of cardiomyocytes. The ability to proliferate markedly decreases towards late gestation whereby subsequent growth of the myocardium occurs mainly by cardiomyocyte hypertrophy. The maturational switch at which time cardiomyocytes no longer divide is species dependent. In rats, cardiomyocytes stop dividing in the first two weeks after birth.

Aim: The aim of this study was to determine the effect of intrauterine growth restriction (IUGR), due to maternal protein restriction, on the number of cardiomyocytes in the rat heart at 4 weeks of age (at the time when the cardiomyocytes have ceased dividing).

Methods: Female WKY rats were fed either a normal protein diet (NPD,20% casein) or low protein diet (LPD, 8.7% casein) during pregnancy and lactation. At 4 week of age, volume and the total number of cardiomyocytes were stereologically determined in male and female offspring.

Results: There was no significant difference in heart volumes between groups, although there was a gender effect ($p = 0.0035$). Likewise there was no significant difference in the number of cardiomyocytes between groups in either the male ($3.50 \pm 0.19 \times 10^7$ and $3.63 \pm 0.18 \times 10^7$) or female offspring ($2.38 \pm 0.20 \times 10^7$ and $2.91 \pm 0.30 \times 10^7$).

Conclusion**:** In this study, we have shown that IUGR did not affect heart size or cardiomyocyte number in 4 week old male and female rat offspring. Since previous studies from our laboratory[1] have shown that the number of cardiomyocytes is reduced in LPD offspring at birth, our findings suggest that there is catch up hyperplasia in the LPD hearts in the early postnatal period.

1. Corstius, H.B., et al., *Effect of intrauterine growth restriction on the number of cardiomyocytes in rat hearts.* Pediatr Res, 2005. **57**(6): p. 796-800

PLACENTAL 11 B HYDROXYSTEROID DEHYDROGENASE 2 AND REGULATION OF FETAL ADRENAL FUNCTION: THE INFLUENCE OF EXOGENOUS GLUCO-CORTICOIDS AND FETAL SEX IN NORMAL AND GROWTH RESTRICTED INFANTS

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Background: Placental inactivation of cortisol by 11β-hydroxysteroid dehydrogenase-2 (11βHSD2) protects the fetus from the deleterious effects of endogenous and exogenously derived glucocorticoid (GC). We have previously demonstrated a sexually dimorphic fetal response to inflammatory stressors in pregnancy. 11βHSD2 was implicated in the mechanisms contributing to the growth reduction observed.

Aim: We aimed to investigate the relationship between placental 11βHSD2 activity and GC exposure, in appropriately grown (AGA) and small for gestational age (SGA) infants with respect to sex.

Method: Placental 11βHSD2 activity was measured by radiometric conversion assay in neonates at 24-28 weeks (n=20), 29-36 weeks (n=20), and 37-42 weeks (n=20) gestation. Umbilical venous cortisol and DHEA were measured by commercial RIA kit. The unpaired *t* test was used for comparison between groups and linear regression used to assess correlation between variables.

Results: Total 11βHSD2 activity (activity rate x placental weight) showed a significant correlation with gestational age (r=0.53, $p<0.0001$) and birth weight percentile (r=0.27, $p<0.05$). Infants exposed to antenatal GC had a higher 11βHSD2 activity rate (479.8 ± 50.3 *vs.* 299.6 ± 48.1 nmol/mg/hr, $p=0.02$), but not total activity ($p=0.06$), than those not exposed. A significant interaction between infant sex, steroid exposure, and total 11βHSD2 activity was evident for the preterm infants (F=4.98, $p=0.03$), with only females exhibiting a significant increase following maternal GC exposure, reaching levels equal to those observed at term. SGA infants had lower 11βHSD2 activity rate (335.2 ± 47 *vs.* 504.4 ± 51 nmol/mg/hr, $p=0.02$), total 11βHSD2 activity (87.1 ± 14 vs. 187.6 ± 29 µmol/hr, $p=0.01$), and umbilical venous cortisol (29.6 ± 6.9 *vs.* 181.7 ± 40.8 nmol/l, $p=0.01$) than AGA infants. Umbilical venous DHEA did not differ between AGA and SGA infants but the ratio of umbilical venous cortisol to DHEA was significantly lower in those infants SGA (1.26±0.5 *vs.* 13.3±6.5, $p<0.05$). In AGA infants not exposed to antenatal glucocorticoids linear regression demonstrated that 11βHSD2 activity, independent of gestation, was a significant predictor of the following measures of neonatal physiological stability: CRIB II ($p=0.01$), mean arterial pressure ($p=0.044$), and systemic vascular resistance ($p=0.043$). These relationships where not observed in infants exposed to antenatal glucocorticoids or born SGA.

Conclusions: The sexually dimorphic response in 11βHSD2 activity following antenatal GC exposure has not previously been reported. Placental metabolism of GC by 11βHSD2 is thought to facilitate the development of an autonomous fetal hypothalamo-pituitary-adrenal axis. Following preterm birth adrenal insufficiency is associated with increased morbidity and mortality. The sexually dimorphic response exhibited by the fetus may explain the increased incidence of poor outcome observed in males. In addition, the altered 11βHSD2 activity observed in SGA infants may affect fetal adrenal function influencing physiological adaptation and contributing to poor neonatal outcome.

EFFECTS OF ETHANOL EXPOSURE DURING LATE GESTATION ON PHYSIOLOGICAL STATUS AND GROWTH IN FETAL SHEEP

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Background: It is well established that prolonged exposure of the fetus to alcohol can result in adverse effects such as growth restriction and neurocognitive impairments. Repeated episodic exposures are common in humans and have been reported to have different effects to those resulting from chronic exposures. In this study, we have examined the effects of daily alcohol exposure over a period of 40 days during the latter half of gestation on fetal physiological status and growth.

Aim: To investigate changes in fetal metabolism and growth in response to repeated alcohol exposure in late gestation.

Methods: Pregnant ewes underwent surgery at 91±1 days of gestational age (GA) to implant carotid artery and jugular vein catheters for blood sampling and infusion, respectively. Daily ethanol infusions began at 95±1 days and continued to 134±1 days, apart from a 3-day break for fetal surgery at 126±1 days. In 5 sheep, 40% ethanol in saline was infused into the ewe over a 1 h period, at dose of 0.75g/kg of maternal body weight; a similar volume of saline was infused into 5 control sheep. At surgery a fetal brachial artery catheter was inserted for blood sampling. At GA 131 to 133 days, blood samples (3 ml) were taken at -1, 0, +1, +2, +4, +6, +8, +10 & +24 hours in relation to ethanol or saline infusions. We measured maternal and fetal plasma alcohol levels (PAC), blood gas tensions, blood glucose and lactate values. Ewes were killed at GA134 days and fetal body and organ weights recorded.

Results: Maximal maternal and fetal PACs of 0.12±0.01 and 0.11±0.01 g/dL respectively occurred 1 hour after the start of the infusion. Maternal blood glucose levels decreased between 1 and 2 hours after the start of the ethanol infusions compared to control ewes. In ethanol exposed fetuses, blood glucose levels decreased compared to controls between 1 and 4 hours after the start of the infusion. Blood lactate increased in ethanol treated ewes from hours +1 to +6 ($p=0.002$), while fetal lactate increased from hours +1 to +10. In ethanol treated fetuses, but not in ewes, SaO_2 decreased ($p=0.005$) from hours +6 to +10; a similar trend was seen for fetal PaO_2. At autopsy (GA134 days) fetal organ and body weights were not different between groups.

Conclusions: Fetal and maternal blood alcohol levels reached maximal values one hour after infusion started. Maternal and fetal blood glucose levels fell transiently after the ethanol infusions. This response could be related to the altered metabolism and/or insulin hypersensitivity in the mother and/or fetus, while elevated blood lactate concentrations are indicative of alcohol metabolism. Reductions in fetal oxygenation could be a result of decreased placental perfusion, resulting in decreased fetal oxygen delivery. Fetal growth showed no evidence of being restricted.

DIETARY CREATINE IN THE PREGNANT SPINY MOUSE (*Acomys cahirinus*) REACHES THE FETUS AND IMPROVES SURVIVAL FOLLOWING BIRTH ASPHYXIA

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Background and Aim: Creatine pre-treatment has been shown to provide neuroprotection against hypoxic-ischemic injury, as illustrated in animal models of neurodegenerative diseases, traumatic brain injury, and stroke, as well as in vitro studies with rat hippocampus and brainstem. A major limitation of creatine treatment in the adult is the limited capacity for exogenous creatine to pass the blood brain barrier and increase the cellular pool of creatine and phosphocreatine (PCr). In both the rat and rabbit, the immature neonatal brain appears to have a much greater capacity for exogenous creatine uptake than the adult brain. We hypothesised that a maternal diet supplemented with creatine would promote the accumulation of creatine/PCr in fetal and placental tissues, thereby preventing or reducing behavioural deficits that occur as a result of an acute asphyxic insult at birth.

Method: Pregnant spiny mice were fed either a standard diet (n=17) or a diet supplemented with 5% creatine monohydrate (n=11) from day 20 (~0.5) of gestation. On day 37 of gestation, 4 dams from each group were killed, and placentas and maternal and fetal brains, hearts, livers and kidneys were dissected, snap frozen and enzymatically assayed for total creatine content (creatine + PCr) using fluorometric detection. For a second cohort (control diet, n=13; creatine diet, n=7), the pregnant uterus was excised and placed in a saline bath at 37°C for 7.5-8mins to induce global asphyxia. Fetuses were expelled and resuscitation attempted. Surviving neonates were cross-fostered to lactating dams and their behaviour assessed between postnatal days 1-15 using the open field test, accelerating rotarod and footprint pattern.

Results: In 13 dams fed the control diet, 24 out of 38 pups (63%) survived the asphyxic insult, whereas for 7 dams fed the creatine diet, 19 out of 20 pups (95%) survived ($p<.005$, one-tailed t-test). The maternal creatine diet produced a significant increase in the total creatine content of placenta ($p<.001$), fetal heart ($p<.05$), and fetal and maternal liver and kidney, when compared to control fed animals and offspring. The asphyxic insult at birth produced significant behavioural abnormalities in offspring assessed between postnatal days 1-15. When compared to vaginally delivered neonates, those exposed to birth asphyxia showed a reduction in spontaneous locomotion, balance and motor coordination. The maternal creatine diet did not prevent these behavioural deficits.

Conclusion: A maternal diet supplemented with 5% creatine monohydrate from mid-gestation resulted in creatine loading in placental and fetal tissues, improved the fetuses' capacity to survive an asphyxic insult at birth, but did not alleviate behavioural abnormalities that emerged in early postnatal life due to the asphyxia. Creatine is promising as a prophylactic therapy for pregnancies classified as high-risk for fetal hypoxic-ischemia, and further investigation into the mechanisms by which creatine may improve neonatal well-being is warranted.

Notes

DOES ANTENATAL MATERNAL STRESS AND ANXIETY INFLUENCE FETAL BEHAVIOUR AND INFANT DEVELOPMENT?

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Aim: To examine the effects of maternal anxiety in pregnancy on fetal behaviour and infant development at 18 months.

Methods: We measured maternal anxiety, using the STAI (trait and state), stress using the perceived stress Scale (PSS) and a Life events stress (LES) scale and depression using the Edinburgh Scale (EPDS), at 30-32 (time 1) and 36-36 wks of gestation (time 2). Fetal heart rate (HR) responses to repeated vibro-acoustic stimulation were assessed at 36-38 wks. Infant development was evaluated by an independent observer at 18 months of age using Bayley Scales of Infant Development (BSID).

Results: Regressions which attempted to explain fetal heart rate changes by maternal stress and anxiety measures were performed in 108 mother-fetal diads. The dependent variables used were: Mean HR (bpm) during baseline, standard deviation of HR (bpm) during baseline: maximum HR change (bpm) following the first 3 stimuli, time to maximum HR change following 1st 3 stimuli (seconds).

The explanatory variables used were the EPDS and STAI-trait total score at times 1 and 2, STAI-State total score at time 2, PSS total score, life event stressors at time 2, fetal weight; maternal ACTH, cortisol and CRH at time 1.

Each of the time 1 explanatory variables was regressed against each of the time 1 stimulation outcomes, and similarly for time 2. No significant results were found, i.e. none of the maternal anxiety and stress measures, including blood hormone levels, were found to be predictive of fetal response to repeated vibro-acoustic simulation.

Seventy-one infants were tested using the BSID scales. Infants whose mothers had STAI scores >45 at both times 1 and 2 had a significantly lower Mental Developmental Index (MDI 111.47 v 116.75 p=0.001) and a lower Behavioural Rating Scale (103.22 v 108.14 p=0.056). The Psychomotor Development Index (PDI) was similar for both groups

Conclusions: Results suggest that maternal anxiety in pregnancy does not impact on fetal behaviour as measured by heart rate responses to repeated vibro-acoustic stimulation. However maternal anxiety does seem to have a significant impact on infant development.

MATURATIONAL CHANGES IN INFANT AROUSAL PROCESSES DURING THE FIRST SIX MONTHS OF LIFE

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Background: Arousal from sleep has been described as a vital protective mechanism against cardiorespiratory failure and a failed or inadequate arousal response is thought to be a factor in the pathogenesis of Sudden Infant Death Syndrome (SIDS). Recently, an International Work Group published a definition of infant arousal which classified arousals into sub-cortical activations (defined by the presence of heart rate, respiratory and behavioural changes); and cortical arousals (which also include a change in electroencephalogram (EEG) frequency).[1]

Aim: The aim of this study was to investigate maturational changes in both induced and spontaneous arousal pathways throughout the first six months of life and how these might be affected by sleeping position. We hypothesized that cortical arousals would be impaired at 2-3 mo, the age of peak SIDS incidence, and that this impairment would be more marked in the prone position, the major risk factor for SIDS.

Methods: Thirteen healthy term infants were studied using daytime polysomnography at 2-4 wk, 2-3 mo and 5-6 mo postnatal age. All infants slept in both prone and supine positions. During active sleep (AS) and quiet sleep (QS) a pulsatile jet of air to the nostrils was used to induce arousal. Arousals which were observed during uninterrupted sleep between tests were classified as spontaneous arousals. Physiological and EEG changes were visually assessed. For induced and spontaneous arousals, cortical arousals were expressed as a percentage of the total sub-cortical plus cortical arousals. Data were compared with Chi-square tests to assess the effects of postnatal age and sleeping position.

Results: A total of 2419 air-jet stimuli produced 902 arousal responses (503 in AS, 399 in QS) for analysis. A total of 526 spontaneous arousals (456 in AS, 70 in QS) were also analysed.
Induced Arousals: In the supine position, during both AS and QS, the proportions of cortical arousals and sub-cortical activations remained similar across all three ages studied. In contrast, when infants slept prone, in both sleep states a significant age-related increase in the proportion of cortical arousals was observed at 2-3 months (AS: 51%, $p<0.05$; QS: 55%, $p<0.01$) when compared with both 2-4 weeks (AS: 34%; QS: 31%) and 5-6 months (AS: 30%; QS: 24%).
Spontaneous Arousals: When infants slept supine, there were no maturational changes in the proportion of arousal types observed across the three ages studied. During AS, when prone, proportions of cortical arousals were significantly increased at 2-3 months (70%) compared with 5-6 months (41%, $p<0.05$). A similar trend was evident during QS however this failed to reach statistical significance ($p>0.05$).

Conclusions: The similar trends observed in both induced and spontaneous arousals support previous studies which suggest that both are generated by the same neural pathway. Additionally, we have shown that the prone position alters arousal pathways to promote full cortical arousal at 2-3 mo, the age where SIDS incidence is highest. We propose that in healthy infants, this may be a protective mechanism during a vulnerable period of development which may fail in SIDS.

1. The International Paediatric Work Group on Arousals. *The scoring of arousals in healthy term infants (between the ages of 1 and 6 months).* J Sleep Research, 2005. **14**: 37-41.

CARDIOVASCULAR CONTROL DIFFERS IN SLEEP IN INFANTS WITH A HISTORY OF APNOEA OF PREMATURITY.

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Background and Aim: Infants born prematurely are at increased risk of cardiovascular instability due to the immaturity of autonomic control of the cardiovascular system. This instability is most marked during sleep, and may play a role in the increased incidence of Sudden Infant Death Syndrome (SIDS) in this group. Apnoea of prematurity is a common complication of prematurity occurring in around 85 % of infants born at <34 weeks gestational age (GA)[1], which been associated with altered heart rate (HR) control[2]. However, little is known about the effects of apnoea on blood pressure (BP) control, particularly after term equivalent age. We aimed to assess the effects of apnoea of prematurity on cardiovascular control.

Methods: 16 infants born between 28-32 wk GA were studied. The infants were divided into two groups based on their neonatal history and severity of apnoea of prematurity and respiratory illness. The numbers of apnoeas (A2) and bradycardias (B2) which required nursing stimulation or supplemental oxygen from birth to discharge home were recorded for each infant. The apnoea group (n=8) had ≥10 A2+B2 and/or needed resuscitation for ≥1 severe events and/or CPAP treatment for apnoeas and/or required >24hr of respiratory support after birth. These infants had Apgar scores of 5-9 at 5min and birth weights of 841g - 1475g. Infants in the control group had <10 A2+B2 and required no additional ventilation. Apgar scores were 6-9 at 5min and birth weights 1080 - 2061g. Both groups of infants were studied using daytime polysomnography at 2-3 wks corrected age (CA). Continuous BP measurements were obtained using the Finometer™ (FMS, The Netherlands) with a cuff placed around the infant's wrist. All infants slept supine and HR and BP data were collected in active (AS) and quiet sleep (QS).

Results: Infants in the apnoea group had significantly lower HR than the control group in both AS and QS. Infants in the apnoea group also had significantly higher mean (MAP) and diastolic (DAP) BP during AS when compared to the control group (Table).

	HR (bpm)	HR SD	MAP (mmHg)	SAP (mmHg)	DAP (mmHg)
Apnoea QS	136 ± 4*	6 ± 1	70 ± 4	87 ± 5	62 ± 4
Control QS	149 ± 2	4 ± 0	68 ± 5	89 ± 6	58 ± 4
Apnoea AS	137 ± 3*	8 ± 1	94 ± 6*	111 ± 6	81 ± 5*
Control AS	147 ± 3	6 ± 1	74 ± 6	94 ± 8	64 ± 5

*Mean ± SEM *p<0.05 Apnoea group vs. control group*

Conclusions**:** This study provides preliminary evidence that a neonatal history of apnoea of prematurity may be associated with alterations in HR and BP control after term equivalent age. These alterations may signify that this group of infants is at greater risk for cardiovascular instability, particularly during active sleep. Study of infants with a history of apnoea and bradycardia at later ages will provide information on the long-term cardiovascular effects of apnoea of prematurity in these infants, particularly at 2-3 mo CA when the risk for SIDS is greatest.

1. Barrington K. and N. Finer. Pediatric Research, 1991. **29:** 372-375.
2. Henslee J., et al., Early Human Development, 1997. **47**: 35-50.

MICRO-RNAS ARE DIFFERENTIALLY EXPRESSED BETWEEN MID AND LATE GESTATION IN THE MOUSE PLACENTA.

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Background and Aim: MicroRNAs (miRNAs) are short, single-stranded, non-coding RNAs involved in the post-transcriptional repression of gene expression. MiRNAs bind to complementary sites in the 3'UTR of target mRNAs to repress or silence translation, which subsequently reduces transcript levels. Placental functional development is characterised by dynamic and co-ordinated changes in expression of structural and functional genes, as invasion, differentiation and growth occur throughout gestation. These may arise in part from miRNA regulatory networks. MiRNAs have been detected in the mammalian placenta but their patterns of expression throughout pregnancy have not been systematically characterised.

Methods: To characterise miRNA expression in the developing placenta, miRNA microarrays were used to compare miRNA gene expression in mid (day 13) and late gestation (day 18) murine placenta (term ~ 21 days). Each miRNA microarray contained 380 probes printed in triplicate, including 3 negative controls.

Results: Approximately 26% of all miRNAs examined were significantly upregulated and ~16% were significantly downregulated in the placenta in late compared to mid gestation ($p < 0.05$). Some of the most highly differentially expressed upregulated miRNAs include: mmu-mir-424, hsa-mir-424, mmu-mir-291-3p and mmu-mir-294. Some of the most highly differentially expressed downregulated miRNAs include: hsa-mir-21, hsa-mir-494, hsa-mir-142-3p and mmu-mir-130a. Many upregulated miRNAs are members of polycistronic clusters, including several from an imprinted cluster found on human chromosome 14q32 and mouse chromosome 12 (mir-127, 136, 154, 410, 376a, 377). The miRNAs in this latter imprinted cluster are maternally-expressed and previous studies have shown that disruption of their expression can lead to impaired placental vascular development, disrupted labyrinth and junctional zone development and embryonic lethality.

Conclusions: Many of the upregulated miRNAs and several of the downregulated miRNAs in the developing mouse placenta are predicted to target genes that regulate trophoblast differentiation and growth, such as CDX-2 and IGF-2 or that are involved in solute transport such as GLUT1. These findings suggest that miRNAs and the factors that influence their expression may have role in the regulation of the functional development of the placenta and hence the fetal environment.

IGF-II SYNERGISES WITH LOW OXYGEN TO PROMOTE PLACENTAL TROPHOBLAST INVASION

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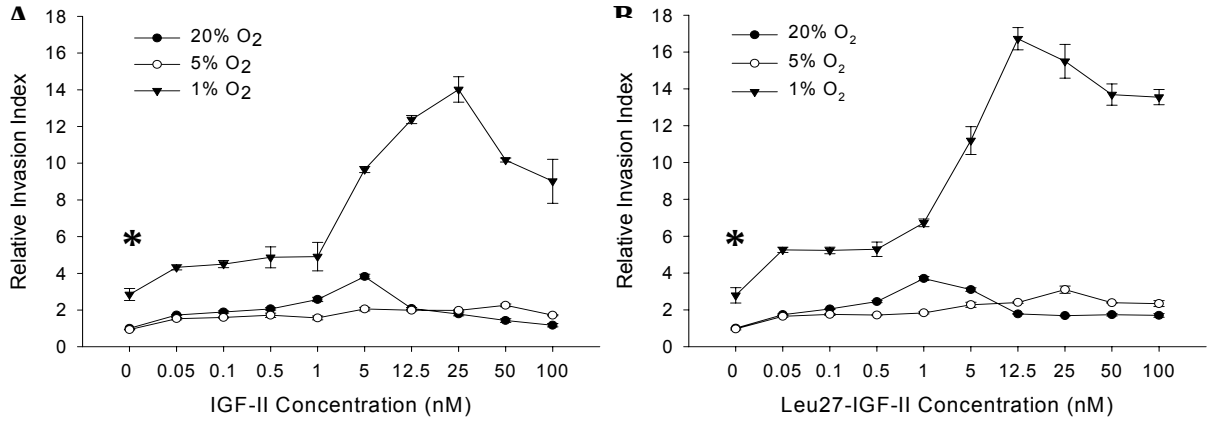
Background and Aim: The major complications of pregnancy, preeclampsia, pre-term birth and intrauterine growth restriction (IUGR), occur in 19% of first pregnancies and often occur together within the same pregnancy. They are life-threatening to either the mother or her baby in more than 6% of pregnancies. Impaired trophoblast invasion has been implicated in these and other complications of pregnancy and results in insufficient remodelling of the spiral arterioles and poor blood flow to the placenta. Paradoxically, up to 10-12 weeks gestation in women the placenta develops in a low oxygen environment, the invading trophoblasts having initially occluded the spiral arterioles that they will subsequently remodel. This physiological but hypoxic environment is thought to be essential for pregnancy success. IGF-II is most abundantly expressed at the feto-maternal interface *in vivo* and has been shown previously to promote trophoblast invasion *in vitro*. However, there are no reports of the effect of oxygen tension on its actions.

Methods: HTR-8/SVneo trophoblast cells were grown on Matrigel coated porous membranes (50,000 calcein-AM labelled cells in 50μl media in AC96 NeuroProbe A series 96-well Boyden Chambers) and allowed to invade through the Matrigel to the underside of the filter for 6 hours. Cells on the upper surface were scraped off and a relative invasion index measured using a fluorescent plate reader to quantify fluorescence. Invasion assays were performed treating HTR-8/SVneo trophoblast cells with 0-100nM IGF-II or LEU27-IGF-II (binds to IGF2R selectively) in 20%, 5% or 1% oxygen.
Results: Culture in 1% O_2 increased invasion by 200% compared to 20% and 5% O_2. The addition of 5nM IGF-II to 20% O_2 cultures increased invasion by about 200%. However, the addition of IGF-II to 1% O_2 cultures dramatically increased invasion to a maximum 14-fold. The effect of treatment with LEU27-IGF-II, an IGF2R selective analogue, was similar but more potent than IGF-II with invasion increasing nearly 17-fold maximally.



Conclusions: Our data suggest that the low oxygen environment of the first trimester placenta stimulates trophoblast invasion of the decidua. Since LEU27-IGF-II selectively binds IGF2R, and not IGF1R nor insulin receptors, the stimulatory action of IGF-II on cells grown in 1%, 5% and 20% O_2 must be mediated through the IGF2R, challenging the dogma that this receptor is merely a sink for eliminating excess IGF-II. The latter clearly synergises with other factors in the low oxygen environment to dramatically potentiate its effects on invasion. The identity of these factors is currently under investigation.

ACUTE EFFECTS OF MATERNAL IGF SUPPLEMENTATION ON PLACENTAL GENE EXPRESSION, TRANSPORT AND NUTRIENT PARTITIONING IN THE GUINEA PIG

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Background and Aims: Placental transfer is a major determinant of nutrient partitioning between the mother and conceptus and hence, of pregnancy success. Previously we have shown that, in the guinea pig increased maternal circulating insulin-like growth factor (IGF) abundance in early to mid pregnancy enhances placental delivery of glucose and total amino acids near term, increasing fetal development and viability (Sferruzzi-Perri *et al*., 2006a and b). In the case of IGF-II, but not IGF-I, effects on placental transport may have been secondary to effect of the treatment on placental structural differentiation near term. To further dissect the IGF specific pathways during pregnancy, we aimed to determine the acute effects of such treatments on placental gene expression and glucose and amino acid transport and nutrient partitioning in mid gestation.

Methods: Pregnant guinea pigs were infused with IGF-I, IGF-II (both 1mg/kg/day) or vehicle subcutaneously from day 20 to 38 of pregnancy and maternal tissue uptake and placental transfer of the non-metabolisable radioanalogues [^{3}H]-methyl-D-glucose (MG) and [^{14}C]-amino-isobutyric acid (AIB) and plasma metabolite concentrations in mother measured on day 35 of pregnancy (term=70 days). Placental expression of the system A amino acid and glucose transporters, *Slc38a2* and *Slc2a1* respectively*, Igf1, Igf2* and vascular endothelial growth factor, *Vegf,* were quantified by reverse transcription Real Time PCR. Effects of treatment on fetal placental parameters were analysed with a linear mixed model, repeated measures ANOVA, with Bonferoni Post Hoc, using number of viable pups as a covariate where required. Maternal parameters were analysed using Univariate ANOVA with Bonferoni Post Hoc tests. Differences between treatments were considered to be significant if $p<0.05$.

Results: IGF-I, but not IGF-II, increased placental and fetal weights (+13%, p=0.036 and +11%, p=0.048, respectively) and MG and AIB uptake by the placenta (+42%, p=0.036 and +68%, p=0.005, respectively) and fetus (+59% and +90%, respectively, both p=0.004). IGF-I treatment induced placental expression of the system A amino acid transporter, *Slc38a2* by nearly 8-fold (p=0.027), without altering expression of the glucose transporter gene, *Slc2a1*. IGF-I treatment reduced placental gene expression of *Igf2* (-51%, p=0.032), while *Igf1* and *Vegf* were unaffected by maternal IGF supplementation. In the mother, IGF-I increased MG and AIB uptake by muscle in mid-gestation ($p<0.05$), without affecting maternal muscle mass or circulating metabolite concentrations.

Conclusion: In conclusion, increased maternal IGF-I, but not IGF-II, exposure during early to mid pregnancy acutely increases placental delivery of glucose and amino acids to the fetus, the latter of which is likely to be due to effects on placental amino acid transporter gene expression. This occurs despite increased maternal nutrient utilisation, implicating IGF-I in the maternal response to pregnancy. Although there was no acute effect of maternal IGF-II supplementation further molecular analyses may help to identify events occurring during treatment which induce the sustained effects of IGF-II on the fetus and placenta near term (Sferruzzi-Perri *et al*., 2006a and b).

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Notes

THE EFFECTS OF INTRA-AMNIOTIC UREAPLASMA INJECTION ON GAMMA-DELTA T CELLS FROM THE THYMUS AND SPLEEN OF FETAL SHEEP

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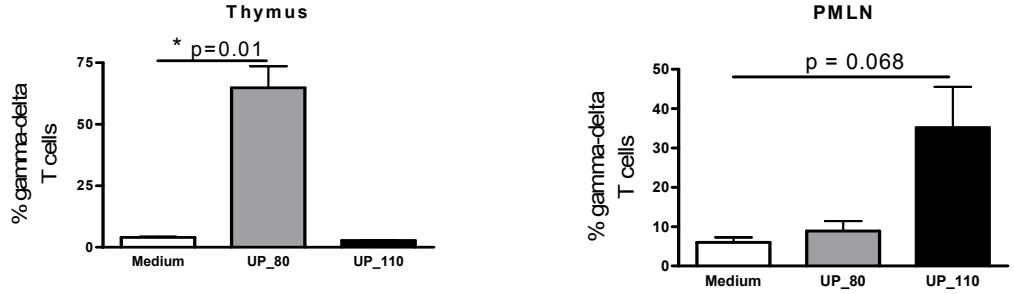
Background and Aim: The fetus is capable of mounting sophisticated immune responses despite an immature immune system, but fetal immune responses can predispose to subsequent allergies. Chorioamnionitis (inflammation of the placental membranes and amniotic fluid) is frequently associated with adverse pregnancy outcomes, including bronchopulmonary dysplasia. We hypothesized that chorioamnionitis induces fetal innate immunity, in turn reprogramming postnatal lung responses.

Methods: Using an established model of chorioamnionitis, ewes were given an intra-amniotic (IA) injection of 10^7 c.f.u. *Ureaplasma parvum* at 80 days of gestation (n = 3) or 110 d (n = 4), or an IA injection of an equivalent volume of saline at 80 d gestation (n = 3). At near-term (145 +/- 3 d), ewes and lambs were euthanised. The thymus and posterior mediastinal lymph node (PMLN) were removed and weighed and mechanically dissociated into single-cell suspensions. The cells were stained with ovine specific antibodies to CD8, CD4, CD25 and T-cell receptor 1 (gamma-delta T cells), and analysed by flow cytometry.

Results: The percentages of T cells from the fetal thymus or spleen that expressed CD8, CD4 and CD25 were not different between ureaplasma and saline groups. In contrast, the proportion of gamma-delta T cells in the fetal thymus and PMLN were higher in the ureaplasma group than control, but this effect depended on the time of injection of ureaplasma.



Conclusions: The increase in percentage of gamma-delta T cells after an infection supports the hypothesis that chorioamnionitis induces fetal innate immunity. The time of exposure to an infection influenced the distribution of gamma-delta T cells in different lymphoid organs, highlighting the complex functionality of these cells in the innate and adaptive immune systems.

Support: Asthma Foundation WA, NIH AI069716-01, NHMRC 404102 & 303261

IMPACT OF NEONATAL ENDOTOXIN EXPOSURE ON LATER LIFE ANXIETY-LIKE BEHAVIOUR IN RODENTS

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Background and Aim: Perturbations in the stress regulatory system (i.e., the HPA axis) are known to play a major role in the aetiology of anxiety disorders. Growing research indicates that such alterations can occur as a consequence of early life epigenetic programming. Animal research for instance has demonstrated that neonatal exposure to stress alters the long term functioning of the HPA axis. Subsequent research has demonstrated that this dysregulation can result from exposure to bacteria early in life, a common neonatal stressor. The present study assessed the effect of neonatal endotoxin exposure on the predisposition, in adulthood, toward anxiety-like behaviours in Wistar rodents.

Methods: Rats were administered either endotoxin (Salmonella enteritidis, 0.05mg/kg, i.p.) or saline (equivolume) on days 3 and 5 of life. In adulthood (day 90) animals underwent a chronic stress paradigm over 3 days. On the first and second days animals were exposed to 30 minutes restraint stress, and on the third day animals underwent 30 minutes of isolation housing. Stress-related activity (resistance within the restraint chamber) was measured as an indication of stress-induced helplessness. Behavioural testing commenced on day 4. Subsets of animals were assessed on either a) the Hidebox-Open Field test, or b) the Acoustic Startle Response (ASR) test. Animals were placed within a hidebox in the Open Field arena and allowed to explore for 15 minutes. The ASR test involved presentation of 150 startle bursts at 108dB for 40 milliseconds with an interstimulus interval of 6 seconds. Animals underwent baseline ASR testing 2 weeks prior to stress, and were tested 4 times over 7 days following stress.

Results: Analysis of the stress-related activity data revealed that endotoxin-treated animals spent significantly ($p<0.05$) less time resisting restraint compared to saline controls possibly indicative of increased susceptibility to helplessness behaviour (i.e., immobility). Furthermore, the impact of neonatal endotoxin exposure on increased helplessness behaviour appears to be sex dependent with a greater effect apparent in females. Endotoxin-treated animals exposed to the Hidebox-Open Field spent more time inside the hidebox, and less time in the centre of the open field compared to controls, suggestive of increased anxiety ($p<0.05$). These animals also exhibited a reduction in exploratory behaviour with fewer movements between the hidebox and open field. Similarly, endotoxin-treated animals spent significantly more time poking their heads out of the hidebox door, suggesting hypervigilance, which is commonly observed in patients with anxiety disorders. Peak values of the startle response of animals on the first and last 10 trials of the ASR test, indicated that endotoxin-treated animals habituated significantly less to the stimulus compared to saline-treated controls.

Conclusions: These results suggest that neonatal exposure to bacteria can produce long-term alterations to behaviour, with a bias towards increasing anxiety-like behaviours in adulthood.

ANTI-INFLAMMATORY EFFECTS OF SULFASALAZINE IN AN OVINE MODEL OF *IN UTERO* INFECTION: A PRELIMINARY INVESTIGATION

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Background: There is evidence to suggest that a causal relationship exists between inflammation arising from intrauterine infection and injury to the fetal brain. Preterm ovine fetal exposure to lipopolysaccharide (LPS) is a commonly used model for exploring the mechanisms underlying inflammation-induced white matter injury. LPS is a potent inflammatory trigger that mimics infection because it initiates components of the mammalian innate immune response, including the stimulation of pro-inflammatory cytokines, such as tumour necrosis factor-α (TNF-α), that are thought, at least in part, to contribute to white matter injury in the fetal brain. Some of the proinflammatory cascade induced by LPS is mediated by the transcription factor nuclear factor kappa-B (NFκB).

Aim: The aim of this study is to investigate the effects of sulfasalazine (SSZ), an NFκB inhibitor, on LPS-induced inflammation and subsequent white matter injury, as a possible clinical therapy for intrauterine infection.

Methods: At 104-106 days gestation (term ~147 days) 5 twin-pregnant ewes underwent surgery to insert catheters into the femoral artery and vein of each fetus. The day prior to surgery, the ewes were randomly assigned to receive SSZ (4g/ day for 3 days and then 2g day thereafter; (n=2) or a placebo (n=3). In each pregnancy, one of the catheterised fetuses received three daily i.v. infusions of LPS (100ng/kg) and the other saline injections of identical volume (LPS alone or saline alone [n=3 each];LPS-SSZ or saline-SSZ [n=2 each]).Fetal blood-gases and pH were monitored daily and plasma samples were taken to measure TNF-α. At the end of the experiment the fetal brains were transcardially fixed with 4% paraformaldehyde for immunohistochemical evaluation of apoptosis (by TUNEL) in the subventricular white matter.

Results: Levels of TNF-α were undetectable in the fetal circulation in both saline alone and saline-SSZ animals. There was an increase in plasma TNF-α concentration (from undetectable levels to 84.4±13.5 ng/mL) in the LPS alone fetuses following the first LPS administration, which returned to baseline within 6 hours post-LPS exposure. There was an attenuation of the TNF-α response with subsequent LPS exposure. Maternal treatment with SSZ significantly mitigated, but not ablated, the LPS-induction of TNF-α (42.2 vs. 84.4ng/mL). No TUNEL positive cells were detected in the subventricular white matter of saline alone or saline-SSZ fetuses. TUNEL-positive staining cells were abundant in the subventricular white matter of the LPS-alone fetuses and present but less abundant in the LPS-SSZ fetuses.

Conclusion: Maternal sulfasalazine can mitigate, but not prevent, LPS-induction of TNF-α in the fetal circulation and LPS-induction of TUNEL positive cells in the subventricular white matter. These data suggest that anti-inflammatory agents, such as SSZ, may be useful neuroprotectants in intrauterine infection.

ERYTHROPOIETIN AS A POTENTIAL THERAPEUTIC AGENT IN THE LPS-MODEL OF FETAL BRAIN INJURY: EFFECTS ON THE PLACENTA AND LIVER

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Background**:** Intrauterine infection is associated with brain injury and preterm birth. Repeated acute exposure to the bacterial endotoxin, lipopolysaccharide (LPS), has been shown to cause fetal hypoxemia, hypotension and white matter injury in fetal sheep at 0.65 of gestation (Duncan et al., 2002). Despite extensive trials of neuroprotective agents, there have been no successful treatments that alleviate the brain injury induced by intra-uterine infection. We have recently found that treatment with recombinant human erythropoietin (rhEpo) appears to attenuate the extent of brain injury in some but not all fetuses exposed to LPS (Rees et al., 2007). In the present study we examined the effects of LPS on the placenta and fetal liver, and tested the protective effects of erythropoietin.

Objective**:** To assess (a) placental and liver structure following LPS-exposure at 0.7 gestation, and (b) the effect of rhEpo treatment on the extent of placental and liver damage.

Methods**:** At 100±2 days of gestation (term ~147 days) ewes and fetuses were anaesthetized and chronically catheterized. Following 5±1 days of recovery, fetuses received on 3 consecutive days either: 1) LPS (~ 0.9µg/kg) followed one hour later by rhEpo (5000 IU) (n=10); 2) LPS alone (n=5); 3) rhEpo alone (n=2); 4) saline alone (n=4). Fetal arterial blood gases and mean arterial pressure were monitored. At 115±2 days of gestation fetuses were euthanised and the fetal brain and other major organs were collected, weighed and prepared for histological assessment. Placental structure was assessed with a quantitative morphometric analysis. Liver structure was assessed qualitatively.

Results**:** Fetuses treated with LPS alone became hypoxemic, acidemic and hypotensive. Treatment with rhEpo did not improve these physiological changes and in some cases they were exacerbated. In the placentomes tissue degeneration and calcification were found in 3/5 LPS-exposed fetuses. Minor damage was found in 3/6 LPS + rhEpo treated fetuses; 3/6 sustained no apparent damage. The extent of placental damage, as a percent of total cross sectional area of the placentome, did not correlate with the severity of brain injury or changes in fetal physiological parameters such as oxygen saturation. There was severe necrotic damage in the liver in 50% of LPS-exposed fetuses; LPS + rhEpo treatment appeared to protect the liver from this form of damage.

Conclusion: rhEpo treatment appears to ameliorate LPS-induced placental and liver damage, but does not improve the physiological status of the fetus. RhEpo might exert its protective effect on the placenta and liver, as well as the brain, via pathways other than improved blood gases. Before rhEpo can be trialled as a neuroprotective agent in 'at risk' infants, further investigation needs to be undertaken on mechanisms by which rhEpo exerts its effects. One possibility is that it occurs via the down-regulation of pro-inflammatory cytokines; this will be examined in future studies.

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