THE TWENTIETH NATIONAL WORKSHOP ON FETAL AND NEONATAL PHYSIOLOGY

Rottnest Lodge, Rottnest Island, WA
April 7 - 8, 2006

Organising committee:

Tim Moss, University of Western Australia
Megan Probyn, Monash University
Richard Harding, Monash University

NGED
ARC/NHRMC Research Network in Genes and Environment in Development

Program

Friday 7th		Saturday 8th	
10.30-11.00 Registration			
SESSION 1		**SESSION 4**	
11.00-11.15	A1	9.00-9.15	A17
11.15-11.30	A2	9.15-9.30	A18
11.30-11.45	A3	9.30-9.45	A19
11.45-12.00	A4	9.45-10.00	A20
12.00-12.15	A5	10.00-10.15	A21
12.15-12.30	A6	**10.15-10.30**	**General discussion**
12.30-12.45	A7		
12.45-1.00	**General discussion**	**10.30-11.00**	**Tea/Coffee**
1.00-2.00	**Lunch**	**SESSION 5**	
		11.00-11.25	A22 Donald Peebles
SESSION 2		11.25-11.40	A23
2.00-2.25	A8 Alan Jobe	11.40-11.55	A24
2.25-2.40	A9	11.55-12.10	A25
2.40-2.55	A10	12.10-12.25	A26
2.55-3.10	A11	12.25-12.40	A27
3.10-3.30	**General discussion**	**12.40-1.00**	**General discussion**
		1.00-2.00	**Lunch and student prizes**
3.30-4.00	**Tea/Coffee**		
		SESSION 6	
SESSION 3		2.00-2.15	A28
4.00-4.15	A12	2.15-2.30	A29
4.15-4.30	A13	2.30-2.45	A30
4.30-4.45	A14	2.45-3.00	A31
4.45-5.00	A15	**3.00-3.15**	**General discussion**
5.00-5.15	A16		
5.15-5.30	**General discussion**		
7.00 Dinner			

Friday 7th April

10.30 -11.00 Registration/Tea/Coffee

Session 1. Chair - Megan Wallace			
11.00	A1	V. Clifton	Sex specific differences in placental gene expression in pregnancies complicated by asthma
11.15	A2	C.T. Roberts	Novel interactions of endocrine IGFs with the placental RAS
11.30	A3 (s)	C.E. Filby	Pulmonary capillary embolisation disrupts alveolarisation
11.45	A4 (s)	F. Sozo	Expression and localisation of thrombospondin-1 in the ovine fetal lung
12.00	A5	S.B. Hooper	Imaging lung aeration at birth: an update
12.15	A6 (s)	B.A. Edwards	Doxapram promotes breathing stability in newborn lambs: a preliminary study:
12.30	A7 (s)	H.L. Richardson	Does swaddling affect arousal responses in sleeping infants?
12.45 -1.00			General Discussion

1.00 - 2.00 Lunch

Session 2. Chair – Vicki Clifton			
2.00	A8	A. Jobe	An expanding translational research agenda in perinatal medicine
2.25	A9 (s)	B.J. Allison	In utero ventilation: a novel tool for investigating neonatal lung injury
2.40	A10 (s)	N.B. Smith	Responses to head-up tilting during sleep in preterm infants
2.55	A11 (s)	M.J. Stark	Characterisation of microvascular function in preterm infants in the first week of life
3.10 -3.30			General Discussion

3.30 - 4.00 Tea/Coffee

Session 3. Chair – Adrian Walker			
4.00	A12 (s)	J.J. Henderson	The effect of antenatal glucocorticoid administration on the endocrinology of lactogenesis II in sheep
4.15	A13 (s)	A.J. Turner	Administration of dexamethasone to the ewe in early gestation using an osmotic pump
4.30	A14 (s)	A.L. Jaquiery	The effect of periconceptional undernutrition on sensitivity of maternal glucose and fat metabolism to insulin in pregnant ewes
4.45	A15 (s)	S.R. Yiallourou	Effect of prone sleeping on blood pressure variability in term infants
5.00	A16 (s)	A.E. O'Connell	Effects of haemorrhage in lambs born to subtotally nephrectomised mothers
5.15 - 5.30			General Discussion

(s) = student

Saturday 8th April

Session 4. Chair – Rosemary Horne			
9.00	A17 (s)	R.A. Corner	The effect of mid-pregnancy shearing on lamb birth weight
9.15	A18	P.R. Kenyon	The effects of the extremes of maternal size within a genotype and maternal nutrition on the resulting offspring
9.30	A19	R.M. De Matteo	Renal and cardiovascular responses in adult sheep exposed to prenatal glucocorticoid treatment
9.45	A20	R. Harding	Does low birthweight due to twinning affect postnatal blood pressure?
10.00	A21 (s)	N. Hodyl	Prenatal immune activation and type of stress exposure: differential effects of stress induced analgesia
10.15 -10.30			General Discussion

10.30-11.00 Tea/Coffee

Session 5. Chair – Frank Bloomfield			
11.00	A22	D.M. Peebles	Comparison of effects of mild hyperthermia or endotoxin on hypoxia induced neural cell death in chick embryos
11.25	A23	E. Stockx	Neuropharmacology of behaviour in the mid gestation fetus
11.40	A24 (s)	P. Cassaglia	The role of the sympathetic nervous system on the cerebral circulation during hypercapnia in sleep
11.55	A25 (s)	Y.S. Feng	Endotoxin effects on cerebral circulation in fetal lambs
12.10	A26 (s)	B. Slattery	Effect of perinatal asphyxia on survival and postnatal behaviour in the spiny mouse
12.25	A27 (s)	B. Slattery	The impact of prenatal stress on hippocampal function in the spiny mouse
12.40 - 1.00			General Discussion

1.00 – 2.00 Lunch and Student Prizes

Session 6. Chair – Tim Moss			
2.00	A28	Z. Kecskes	Protein 14-3-3 in the CSF of newborn rats following hypoxia-ischemia
2.15	A29	B. Lingwood	Cyclical response of newborn piglets to global hypoxia
2.30	A30	G.R. Polglase	Antenatal exposure to inflammation reprograms postnatal airway responsiveness and immune status
2.45	A31	T.J.M. Moss	Effects of medroxyprogesterone acetate on intrauterine inflammation induced by amniotic fluid ureaplasma infection
3.00 - 3.15			General Discussion

(s)=student

A1

Sex specific differences in placental gene expression in pregnancies complicated by asthma

Vicki Clifton[1], Annette Osei-Kumah[1], Isabella Caniggia[2], Igor Jurisica[3] and Nima Soleymanlou[2]

[1]. Mothers and Babies Research Centre, Hunter Medical Research Institute, Newcastle, Australia [2]; Samuel Lunenfeld Research Institute, Mt Sinai Hospital, Canada, [3]Department of Medical Biophysics and Computer Science, University of Toronto, Canada.

Introduction: Previous research examining the effect of maternal asthma during pregnancy on placental function and fetal outcome indicated there were sex specific differences in how the fetus responds to maternal asthma. The female fetus had reduced growth and adrenal function due to alterations in placental glucocorticoid metabolism in response to maternal asthma. Placental function, HPA activity and growth of the male fetus appeared to be unaffected by asthma or inhaled steroid use. However in the presence of a second stressor in the asthmatic maternal system which was an acute, severe asthma exacerbation, males had reduced growth and an increased incidence of stillbirth while the female fetus appeared unaffected. These data suggested male and female fetuses initiate different mechanisms to the same stress and raised the question of whether there were global differences in placental gene expression in relation to fetal sex, maternal asthma and inhaled glucocorticoid treatment.

Methods: Using microarray we determined the gene expression profiles of placentae collected from male or female fetuses of normal, human pregnancies and pregnancies complicated by asthma in the presence and absence of inhaled glucocorticoid intake. Data was analysed using a Binary Tree Structured Vector Quantization algorithm which generates a gene expression map. Sites on the map where there were obvious differences in gene expression were selected for analysis.

Results: Placentae from female fetuses of asthmatic mothers that did not use inhaled steroids during pregnancy had 37 gene alterations relative to the control population. Placentae from male fetuses of asthmatic mothers that did not use inhaled steroids during pregnancy had 6 gene changes relative to the control population. Placentae from female fetuses of asthmatic mothers who did use inhaled steroids during pregnancy had 22 gene alterations relative to the control population and placentae of male fetuses had no gene changes. There were 10 placental genes altered in the presence of maternal asthma that were common to both male and female fetuses.

Conclusions: This preliminary data indicates that there are significant differences in how a placenta from a male fetus and female fetus respond to a maternal disease and raises the question of whether we are significantly compromising our interpretation of human placental data when we do not take the sex of the fetus into account.

A2

Novel interactions of endocrine IGFs with the placental RAS

Standen P[1], Lumbers ER[2], Kumarasamy V[2], Sferruzzi-Perri AN[1], Taylor R[1], Heinemann G[1], Owens JA[1], Roberts CT[1]

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Introduction: Gene ablation studies have shown important roles for IGFs in fetal and placental growth. Exogenous administration of insulin-like growth factors (IGFs) to guinea pigs in early to mid-pregnancy (days 20-38) has sustained effects on placental and fetal growth near term (Sferruzzi-Perri et al. 2006). IGF-II clearly acts through the placenta while IGF-I actions appear to be mediated through the mother. To understand the molecular mechanisms involved we aimed to determine the acute effects of maternal IGF treatment at day 35, on components of the placental renin angiotensin system (RAS) and placental and fetal growth outcomes.

Methods: Guinea pigs (600g) were mated and on day 20 post coitum (pc) mini osmotic pumps were subcutaneously implanted on the back and set to deliver 1mg/kg/day IGF-I, IGF-II or vehicle for 17 days. Guinea pigs were killed at day 35 pc and fetuses and placentas were weighed and either fixed in 4% paraformaldehyde and processed into paraffin or snap frozen in liquid N_2. Total RNA was extracted from frozen placenta with Trizol (Invitrogen), reverse transcribed with Superscript III (Roche) and subjected to quantitative real time PCR using specific primers for transforming growth factor-β1 (TGFβ1), renin, and type 1 angiotensin receptor (AT_1R) mRNAs. Placental sections were stained with Amsson's Trichrome and examined using Video Pro Image Analysis software to measure the total mid-sagittal cross sectional area, and those of the interlobium (germinative region) and labyrinth (exchange region). Double labelling immunohistochemistry followed by point and intercept counting were employed to quantify the proportions of the placental labyrinth composed of trophoblast, fetal capillaries and maternal blood spaces and the surface area and barrier thickness of trophoblast for exchange.

Results: Maternal IGF-I treatment increased placental weight (+12.7%, p=0.036) and fetal weight (+11%, p=0.048). IGF-I also reduced the mid-sagittal cross-sectional area of the exchange region of the placenta (-22%, p=0.014). In contrast, IGF-II had no acute effects on any of these parameters. There were no differences in the morphometry of the placental labyrinth in IGF treatment groups. Both IGFs decreased placental transcription of TGFβ1 by over 77% ($p<0.03$), and IGF-I also decreased placental transcription of AT_1R (-87.8%, p=0.01) but neither IGF altered placental renin gene expression. Excitingly, both IGFs increased the ratio of active to total renin in placental homogenates (IGF-I +49%, p=0.008; IGF-II +39.4%, p=0.015).

Conclusions: We conclude that endocrine IGFs in early to mid pregnancy enhance placental RAS activity possibly by increasing placental protease activity. In early pregnancy, this may be important for placental angiogenesis and the maternal vascular response to pregnancy and act to improve pregnancy outcome. This research indicates novel and interesting mechanisms not previously described in the placenta.

Sferruzzi-Perri AN, Owens JA, Pringle, KG, Robinson JS, Roberts CT, 2006, Maternal insulin-like growth factor-I and –II act via different pathways to promote fetal growth. *Endocrinology* (in press)

A3

Pulmonary capillary embolisation disrupts alveolarisation

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Background: Chronic lung disease (CLD) is a major complication of prolonged ventilatory support in very preterm infants. Lungs of infants with CLD exhibit an arrest of alveolar development, with fewer larger alveoli, and have abnormally developed peri-alveolar capillaries. It has been suggested therefore, that signalling between the developing pulmonary vascular network 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 secondary septation and alveolarisation. Our aim was to investigate the effect of capillary embolisation of the left lung, on fetal lung architecture, secondary septal formation and alveolarisation.

Methods: Pregnant ewes underwent surgery at ~110 days of gestational age (GA; term ~147d) to implant fetal vascular, tracheal, left pulmonary artery and amniotic sac catheters and a transonic flow probe around the left pulmonary artery. At 113-116d GA fetal systemic blood pressure, tracheal pressure, heart rate and blood flow through the left pulmonary artery (PBF) were recorded for 6h. After a 2h control period lung liquid was drained before giving a bolus dose (1 million/ml) of 15μm microspheres into the left pulmonary artery every 10min until a 25% reduction in PBF was achieved (n=3). Control fetuses received vehicle injections (heparinised saline containing 0.01% Tween 80) (n=3). All animals were killed at 130-131d GA. The fetal lungs were removed, each lung was weighed separately before being fixed via the airways at 20 cmH_2O with 4% paraformaldehyde for light microscopy. Using stereological techniques, we determined %-tissue-airspace, alveolar number, secondary crest density, elastin deposition and distribution and erythrocyte number, in control and embolised lungs.

Results: Pulmonary arterial embolisation had no effect on lung wet weight. There was, however, a tendency for the tissue-airspace fraction to increase following embolisation (controls 19.3±1.4% vs. embolised 22.3±1.8%, p=0.13). Although the proportion of lung tissue stained for elastin was not altered by embolisation (controls 4.0±0.9% vs. embolised 4.6±0.7%), its deposition was abnormal, predominantly occurring in the parenchyma and around the base of alveoli, rather than at the tips of developing septal crests. There was no difference in radial alveolar counts between the two groups, however septal crest density was significantly decreased (controls 4.0±0.8% vs. embolised 1.0±0.2, p=0.009) following pulmonary embolisation. The number of red blood cells was reduced by ~21% in embolised lungs (p=0.09).

Conclusions: Our preliminary data has shown that pulmonary arterial embolisation (n=3) has detrimental effects on fetal lung development with dysregulation of elastic fibre distribution and a reduction in the formation of septal crests, which are a hallmark of alveolar development. As elastin deposition at the tips of secondary septal crests is a critical event for alveolarisation, our data suggest that insults to the pulmonary capillary network during development may lead to an arrest of alveolarisation in the developing lung.

A4

Expression and localization of thrombospondin-1 in the ovine fetal lung

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Background: Growth and development of the lungs *in utero* is dependent on the level of distension experienced by the lungs; an increase in lung distension accelerates lung growth, whereas a decrease in lung distension causes lung growth to cease. The mechanisms involved, however, remain unknown. Thrombospondin-1 (TSP-1) was recently identified as a gene up-regulated during accelerated fetal lung growth. TSP-1 is a secreted extracellular matrix glycoprotein that can regulate cell proliferation, cell attachment and spreading, and angiogenesis. As such, we hypothesized that TSP-1 may regulate the growth and development of the fetal lung.

Aims: To determine the expression and localization of TSP-1 during normal lung development and in models of accelerated and retarded lung development in sheep.

Methods: Lung tissue was collected from 90d, 105d, 111d, 128d, 138d and 142d gestational age (GA) fetuses (term ~147d) and 2wk old lambs, as well as from fetuses with accelerated or retarded lung development. Accelerated fetal lung development was induced by increasing lung distension, resulting from tracheal obstruction (TO), for 36h, 2d, 4d or 10d. Retarded lung development was induced by lung liquid drainage (LLD) for 7d or 20d, which reduces lung distension. All experiments were compared to age-matched 128d GA (for TO groups) or 131d GA (for LLD groups) control fetuses (n=4-5 per group). Northern blot analysis was used to determine TSP-1 mRNA levels in all groups, in-situ hybridization was used to examine the localization of TSP-1 mRNA in control fetuses and fetuses exposed to 36h of TO and immunohistochemistry was used to examine the localization of TSP-1 protein in control fetuses and fetuses exposed to 36h and 2d of TO.

Results: During normal lung development, the levels of TSP-1 mRNA were reduced to ~80% of 128d GA values ($p<0.005$) at 138d and 142d of gestation. TSP-1 mRNA levels increased to 347.5$\pm$73.6% of control levels (100.0$\pm$14.0%; $p<0.05$) at 36h of TO but were similar to control levels at 2d, 4d and 10d of TO. TSP-1 mRNA levels were not altered by 7d of LLD but were significantly reduced to 39.4±6.1% of control levels (100.0±20.4%; $p<0.05$) following 20d of LLD. TSP-1 mRNA is expressed predominantly in the nuclei of fibroblasts and type II alveolar epithelial cells within the fetal lung and TSP-1 protein is deposited in most cell types of the lung as well as in the extracellular matrix. The percentage of cells containing TSP-1 mRNA increased ~2.7-fold following 36h of TO ($p=0.005$) and the percentage of lung tissue area containing TSP-1 protein was almost doubled in fetuses exposed to 36h and 2d of TO compared to control fetuses ($p<0.001$).

Conclusions: TSP-1 mRNA and protein levels were increased at 36h and 2d of TO, when cell proliferation rates are high. On the other hand, TSP-1 mRNA levels were reduced during late gestation and at 20d of LLD, when cell proliferation rates are low. Given the localization of TSP-1 within the lung and the various functions it has, we speculate that TSP-1 may have a significant role in regulating fetal lung growth.

A5

Imaging lung aeration at birth: an update

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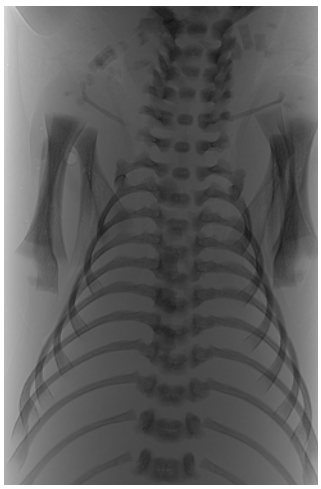
Dept. Physiology[1], School of Physics[2], Dept. Medical Imaging and Radiation Sciences[3] and Centre for Synchrotron Science[4], Monash University; Spring-8 Synchrotron facility[5], Japan and Centre for Accelerator Science, Imaging and Medicine[6], Daresbury,UK.

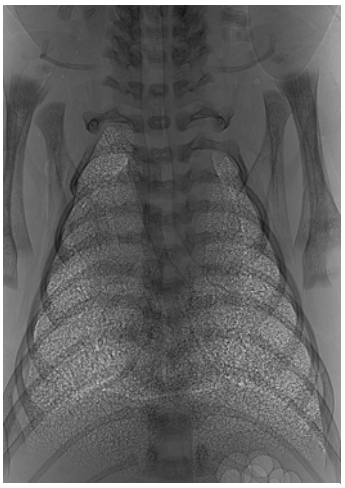
Background: Before birth, the future airways of the lung are liquid-filled, but at birth, this liquid must be cleared to allow the entry of air and the onset of gaseous ventilation. Infants that are born preterm commonly suffer from airway liquid retention which restricts gas exchange and promotes non-uniform pulmonary ventilation, thereby increasing the risk of lung injury. Little is known of the dynamics of lung aeration or the factors that regulate it because of the inability to measure the distribution of air within the lung. We have recently developed an imaging technique, called phase contrast X-ray imaging, that allows us to determine the pattern of lung aeration from birth. A synchrotron, located in Japan, was used as the X-ray source.

Aim: To study the rate and pattern of lung aeration at birth using phase contrast X-ray imaging

Methods: Phase contrast X-ray imaging utilizes differences in refractive index between air and water to image low absorbing structures. After birth, the lung is predominantly comprised of air (~80% by volume) which contrasts strongly with surrounding tissue structures (mainly water), allowing them to be imaged with high contrast and spatial resolution. A Synchrotron (SPring-8, Japan) was used as the radiation source because of its brightness and high beam intensity. At 29-31d GA (term is 32d), pregnant rabbits (n=15) were anaesthetized and pups were delivered by C-section. The pups were either imaged live from birth (n=10) or killed then imaged. Images of live pups were collected at 1-2 sec intervals within the first few breaths of birth. Pups imaged after death were killed after a few breaths, at 3-5mins, 10mins, 15mins, 30mins, 1hr or 2hrs after ventilation onset (n=6 per group); fetuses were also imaged.



Fetus



Neonate after 2h

Results: Phase contrast X-ray imaging can clearly resolve both large (trachea) and small (<100μm) airways of the air-filled lung; liquid-filled fetal lungs are not visible using this technique. Lung aeration at birth spreads rapidly from the large, central airways to the peripheral regions, depending on respiratory activity. The aeration pattern is not uniform as the basal regions aerate last and there is a marked effect of body position; dependent regions aerate at significantly reduced rates.

Conclusions: This technique is ideal for determining the rates and patterns of lung aeration after birth. We are currently developing mathematical models to calculate air volumes and size distribution of respiratory units within specific lung regions.

A6

Doxapram promotes breathing stability in newborn lambs: a preliminary study

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Introduction: Preterm and term infants are prone to breathing instabilities such as apnea and periodic breathing (PB) during sleep. The peripheral chemoreceptors (in particular the carotid bodies) are critical components of the feedback control loop of breathing and are known to play a role in the generation of PB. Doxapram, which appears to mimic the effect of hypoxia on the carotid bodies, is used clinically to stimulate breathing in order to prevent recurrent apnea in preterm infants, however the exact mechanisms are poorly understood.

We hypothesise that doxapram decreases the rate of occurrence of breathing instabilities via increasing the resting drive to breathe.

Methods: We used a newborn lamb model of PB already established in our laboratory in which PB is produced following a period of hyperventilation. In this model we examined the effect of administration of doxapram on the pattern of PB (apnea length, epoch length, cycle number and cycle time), the apneic threshold (level of PCO_2 at which breathing resumes following apnea) and the response to hypoxia. Results are expressed as (mean ± SE).

Results: Following hyperventilation induced apnea in four lambs, a period of respiratory instability was produced when breathing began in $FIO_2 = 0.4$. Doxapram caused a significant reduction in the average PB epoch; 65.8 ± 26.9s to 24.1 ± 9.2sec. ($p \leq 0.05$) as well as a significant reduction in the number of cycles seen; 6.6 ± 2.2s to 2.3 ± 0.7sec. ($p \leq 0.05$). Doxapram significantly reduced the apneic threshold from a PCO_2 of 54.2 ± 2.2 mmHg to 48.3 ± 1.6 mmHg ($p \leq 0.05$). Doxapram shifted the response to isocapnic hypoxia vertically without a change in sensitivity (slope of the ventilation vs. PaO_2).

Conclusions: We propose that doxapram exerts its effect in stabilising breathing by increasing the resting drive to breathing and in effect, by shifting the control curve towards lower PCO_2 values, thereby rendering breathing more stable.

A7

Does swaddling affect arousal responses in sleeping infants?

Heidi L Richardson[1], Stephanie R Yiallourou[1], John Trinder[2], Adrian M Walker[1], Rosemary SC Horne[1]

[1]Ritchie Centre for Baby Health Research, Monash Institute of Medical Research, Monash University and [2]Department of Psychology, The University of Melbourne, Melbourne

Background: Impaired arousal responses from sleep may play an important role in the pathogenesis of Sudden Infant Death Syndrome (SIDS). The major risk factor for SIDS is sleeping prone and previous studies have shown that infant arousal responses are depressed in this position. While SIDS public awareness campaigns have focused on the risks of prone sleeping, some parents continue to sleep their infants prone, reporting that they sleep better that way. To help improve infant sleep in the supine position and thus reduce the risk of SIDS, swaddling or tightly wrapping has recently been recommended by SIDS organisations. This traditional care practice reduces motor activity and favours sleep, though the physiological mechanisms underlying this are uncertain. Previous studies have shown that swaddling reduces spontaneous arousals and increases auditory thresholds during active sleep (AS).[1] However these studies were not performed in quiet sleep (QS), a state where infant arousability is already reduced. The aim of this study was to evaluate the effects of swaddling on infant sleep and arousal to a somatosensory stimulus, during both AS and QS.

Methods: To date, three of a proposed twenty-four healthy term infants have been recruited. Daytime polysomnography was performed at 3-4 weeks and at 3 months after birth; for each study infants slept supine, both unswaddled and swaddled following guidelines recommended by the organization SIDS and Kids. To test arousability, a pulsatile jet of air was delivered alternately to the nostrils in both AS and QS. If the infant failed to arouse, the air pressure was increased at the next stimulus presentation to that nostril; if an arousal was scored, the pressure was decreased. Arousals were scored using criteria as previously described by the Pediatric Workgroup on Arousals.[2] For each nostril, arousal thresholds were calculated as the mean driving pressure of the stimulus between each pair of arousal and non-arousal tests.

Results: Preliminary findings show that at 3-4 weeks, in both AS and QS, mean arousal thresholds are increased when infants are swaddled (154 ± 39 cmH_2O in AS; 307 ± 38 cmH_2O in QS) compared with unswaddled (133 ± 48 cmH_2O in AS; 289 ± 65 cmH_2O in QS). Furthermore, the higher arousal thresholds observed during QS compared with AS are similar to results of previous studies from our laboratory.[3]

Potential Significance: This study will provide new scientific evidence for the mechanisms by which infant swaddling modifies sleep and arousal processes in healthy term infants.

1. Franco P, Seret N, Van Hees JN, Scaillet S, Groswasser J, Kahn A. Influence of swaddling on sleep and arousal characteristics of healthy infants. Pediatrics 2005;115:1307-11.
2. 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 Res. 2005;14:37-41.
3. Horne RSC, Ferens D, Watts A-M, et al. Effects of maternal tobacco smoking, sleeping position and sleep state on arousal in healthy term infants. Arch. Dis. Child. Fetal Neonatal Ed. 2002;87:F100-F105.

A8

The expanding translational research agenda in perinatal medicine

Alan Jobe

Notes….

A9

In utero ventilation: A novel tool for investigating neonatal lung injury

Allison BJ[1], Crossley KJ[1], Flecknoe SJ[1], Morley CJ[2], Harding R[1] and Hooper SB[1].
[1]Dept Physiology, Monash University, Melbourne, and [2]Neonatal Services, Royal Women's Hospital, Melbourne.

Background: Chronic lung disease (CLD) affects ~40% of very preterm infants, is associated with ventilation-induced lung injury (VILI) and is characterized primarily by disrupted lung development. It is difficult to determine which aspects of ventilation cause injury and, in particular the mechanisms by which VILI disrupts lung development. We have recently developed a model of *in utero* ventilation in which the fetal physiological state is maintained by the placenta. This allows the individual causes of VILI to be examined without adverse effects on the fetus.

Objective: To investigate the mechanisms of VILI using *in utero* ventilation in fetal sheep.

Methods: An endotracheal tube was inserted at 105d GA (n=10; term ~147d) in fetal sheep and connected to a saline-filled neonatal ventilation circuit that was exteriorized from the ewe at surgery. At 110d GA, lung liquid was drained from the ventilation circuit and fetuses were ventilated for 12 hrs using 21% oxygen, a PEEP of 4 cmH_2O and a PIP of 40 cmH_2O. The fetuses were either euthanased (n=5) immediately or the ventilation circuit was refilled with lung liquid and normal tracheal liquid flow restored for a further 7 days of *in utero* development (n=5). Following autopsy, lung tissue was examined for lung injury and arrest of alveolar development; unventilated age-matched controls were used for comparison.

Results: *In Utero Ventilation alone (IUV):* After 12hrs of *in utero* ventilation the lungs were hypercellular with prominent areas of non-uniform inflation, resulting in an increase in the percentage of tissue to air space (IUV 65.9 ± 2.5%; control 42.9 ± 2.9%; $p<0.05$), and the future airspaces were simple in structure. The deposition of elastin was increased (3.5 ± 0.4% vs 1.4 ± 0.5% of the tissue) and abnormally distributed compared with control lambs ($p<0.001$). The density of α smooth muscle actin (αSMA) staining within the peri-alveolar parenchyma was increased (16.4 ± 1.3% vs 11.3 ± 1.5% of tissue) and the deposition of collagen I and III was abnormal compared to controls.
In Utero Ventilation with 7d further development (IUVLR): After 12h of *in utero* ventilation followed by 7 days *in utero* development, the lungs had distinct areas of non-uniform inflation and persistent hypercellularity (tissue:airspace; IUVLR 53.0 ± 2.07% vs controls 29.3 ± 9.2% $p<0.001$). The deposition of elastin was also increased (4.3 ± 0.5% vs 1.2 ± 0.9% of tissue) compared with age-matched controls ($p<0.05$) as was the deposition of αSMA as well as collagen I and III.

Conclusions: Ventilation of fetal sheep *in utero* causes morphological changes that are similar to those seen in VILI and CLD. This model has the potential to delineate the individual ventilatory factors that cause lung injury in very preterm infants.

A10

Responses to head-up tilting during sleep in pretem infants

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Background: Preterm infants are at increased risk of cardiovascular instability due to the immaturity of the cardiovascular system and its control by the autonomic nervous system, and this is marked during sleep. We aimed to define the development of autonomic control in preterm infants by assessing mean arterial pressure (MAP) and heart rate (HR) responses to head-up tilting during sleep.

Methods: Eight preterm infants born at 28-32 wks GA with birth weights of 982-1748g were studied longitudinally using daytime polysomnography at 2-3 wks, 2-3mo and 5-6mo corrected age (CA). All infants slept supine and data were collected in both active sleep (AS) and quiet sleep (QS). Infants were tilted head-up by 15° for 30s. Data were analysed beat-beat for 30 and 60 beats following the tilt and compared to 30 beats prior to the tilt.

Results: Sleep state and postnatal age had marked influences on baroreflex responses (BRS) to tilting with MAP responses increasing and HR responses decreasing at both 2-3wks and 2-3mo in QS, whilst results were variable in AS. At 5-6mo, an adult like response, with an initial fall in MAP followed by a rapid increase in HR was observed in QS. Percent change in MAP and HR at the tilt and BRS were not affected by either sleep state or postnatal age.

Conclusion: In this group of healthy preterm infants control of HR and MAP appears to undergo maturation after term until 5-6 mo of age. Further studies are needed to detect any differences from term infants.

A11

Characterisation of microvascular function in preterm infants in the first week of life

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Aim: Male sex is one of the best predictors of death in preterm infants, with preterm males requiring more cardiovascular support than females born under the same conditions. Hypotension occurs in up to 20% of VLBW infants, most commonly in the first 48 hours of life. Abnormal regulation of vascular resistance, with inappropriate microvascular vasodilatation, has a major role in the development of hypotension in preterm infants. The aim of this study was to characterize peripheral microvascular function in preterm neonates in relation to gestation and neonatal sex over the first 7 days of life.

Method: Infants were studied at 24-28 weeks gestation (Group A, n=37) and 29-36 weeks gestation (Group B, n=35). Peripheral microvascular responses were examined using laser Doppler flowmetry on postnatal days 1, 3, 5 and 7. Baseline blood flow and vasodilatory response to Acetylcholine (ACh), heat, and post-occlusive reperfusion where analyzed by unpaired t-test and ANOVA. Results are expressed as mean ± SEM with $p<0.05$ considered statistically significant.

Results: Peripheral microvascular baseline blood flow demonstrated a significant inverse correlation with mean arterial blood pressure (MAP) ($R^2=0.1272$, $p=0.0023$). Baseline microvascular blood flow was significantly higher in Group A on days 1, 3 and 5 (Table 1). Male infants in Group A have a decrease in baseline flow from day 1 to 7 (116.13±19.99 *vs.* 58.79±9.64 PU, $P<0.05$ ANOVA). Female infants did not demonstrate a significant temporal change. Male infants have significantly greater baseline blood flow on day 1 in Group A (116.1±19.99 *vs.* 55.5±7.62 PU, $P=0.008$). This sex specific difference was not apparent by day 3 or present in Group B.

Postnatal Day	Group A (24-28wk)	Group B (29-36wk)	*P*
1	99.29±15.21	36.42±6.89	0.0005
3	72.84±11.18	36.77±7.7	0.01
5	84.73±12.28	31.69±4.31	0.0003
7	60.71±7.64	56.25±11.0	N/S

Table 1. Baseline blood flow (PU) on postnatal days 1, 3, 5, and 7. Mean ± SEM Unpaired t-test Welch correction

No significant gestational age or sex-specific difference was demonstrated in maximal vasodilator response to ACh. Maximal vasodilator response to heat was significantly higher on day 1 in Group A (70.21±10.13 *vs.* 23.45±4.89 PU, $P=0.001$) but was no different from Group B by day 3. No sex-specific differences in heat induced vasodilation where evident.

Conclusion: This is the first detailed characterisation of microvascular function in extremely preterm infants. Abnormal regulation of vascular resistance, producing inappropriate microvascular vasodilatation, has a major role in the development of hypotension in preterm infants. Our findings support a sex-specific disturbance in neonatal vaso-regulation in these high-risk preterm infants that may contribute to the male excess in neonatal morbidity and mortality. Vasodilation in response to ACh and heat did not exhibit sex-specific differences. The mechanisms underlying the observed sex-differences must therefore be separate from the ACh-endothelial pathway.

A12

The effect of antenatal glucocorticoid administration on the endocrinology of lactogenesis II in sheep

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Background and Aims: The onset of copious milk secretion (lactogenesis II) normally occurs at parturition in sheep and is inhibited in pregnancy by high concentrations of progesterone in maternal plasma. Elevated maternal concentrations of the endogenous hormones prolactin and glucocorticoids, and the sudden withdrawal of progesterone just prior to parturition, are required for the onset of lactogenesis II. The aim of this study was to investigate the effects of intramuscular administration of betamethasone to pregnant sheep on the hormones required for lactogenesis II.

Methods: Pregnant ewes bearing single fetuses (N=36) were randomized to receive either betamethasone (Celestone Chronodose 0.5mg/kg IM) at 125 days of pregnancy (d) with medroxyprogesterone acetate 150 mg (MPA) at 118 d (to prevent betamethasone-induced pregnancy loss) (BETA); or MPA at 118 d and saline at 125 d (MPA); or saline at 118 d and 125 d (SAL). Maternal blood samples were collected by jugular venipuncture at 118, 125, 127, 130, 136 and 143 days of pregnancy. Lactogenesis II was determined by changes in the concentration of lactose in maternal blood. A rapid increase in lactose concentration in plasma indicates onset of lactogenesis II. Plasma concentrations of progesterone, cortisol and prolactin were determined using radioimmunoassay (RIA). Data were compared by analysis of variance for repeated measures.

Results: Plasma lactose concentration was significantly increased between two and five days after treatment in the BETA group but did not change in either the MPA or SAL groups (adjusted $P < 0.001$). Plasma progesterone concentration increased over time in all groups but was significantly reduced in the BETA group compared with either the MPA or SAL groups at all time-points after treatment (adjusted $P < 0.001$). Overall there were no changes in plasma cortisol in either the MPA or SAL groups but there was a significant transient reduction in cortisol in the BETA group after treatment (adjusted $P < 0.001$).

Conclusions: Plasma lactose concentration increased dramatically after maternal glucocorticoid administration during pregnancy indicating a precocious onset of lactogenesis II. This was accompanied by a rapid and sustained decrease in plasma progesterone concentration and a transient decrease in plasma cortisol concentration. These findings suggest that, despite the prior administration of MPA, premature lactogenesis II was caused by the withdrawal of progesterone in the presence of uniformly high levels of prolactin and synthetic glucocorticoids.

A13

Administration of dexamethasone to the ewe in early gestation using an osmotic pump.

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Intravenous dexamethasone infusion (0.48mg/hr) to the pregnant ewe for 48hrs during early gestation (26-28 days of gestation, term=150d) is an established model of fetal programming. It has been shown that offspring of such pregnancies have a reduced number of nephrons and are hypertensive as adults (Wintour et al 2003).

We are using this model to study the impact of fetal programming on the kidney at the single nephron level using micropuncture techniques to determine whether the tubuloglomerular feedback system (TGF) is altered during development in this model. TGF is a key mechanism in the regulation of sodium homeostasis. It is thought that abnormalities in the TGF system may lead to the development of hypertension in later life. Given that more than 25% of the population in Australia will develop hypertension during adult life, it is of great significance to determine the underlying mechanisms involved in fetal programming leading to development of this disease. Our studies are being conducted in fetal sheep aged 137-141 days of gestation (term=150 days), and lambs at 8-17days of age.

The published method of delivery of the dexamethasone (Dodic et al 1998) involves long line jugular catheters which must be maintained during a 48hour infusion. We have developed an alternative method of infusing dexamethasone to pregnant ewes at 26-28 days of gestation using an osmotic infusion pump (Alzet) placed subcutaneously, with a catheter inserted in the tarsal vein in the lower leg. The pump (calibrated to pump at 10ul/hr) is filled with saline and a length of PE catheter tubing (id 0.75mm x od 1.45mm) containing the dexamethasone (23.04mg dexamethasone in 0.48ml water) is attached to the pump. The catheter is 1.086m long, and is coiled around the pump before placement. The catheter is inserted under local anaesthetic (lignocaine 1g, ASTRA, Australia) and the wound closed. The surgical procedure takes approximately 30 minutes. The pumps are removed 2 weeks later. In late gestation the pregnant ewes are transported to our laboratory and the fetuses and newborn lambs are prepared for renal micropuncture studies.

Blood pressure will also be measured in a cohort of offspring of the dexamethasone treated ewes at the age of 6 months. This is to confirm that the osmotic pump, intravenous method of administration of the dexamethasone causes a similar increase in blood pressure as has been published using the jugular vein cannulation technique.

Wintour *et al* 2003 – Journal of Physiology (2003) 549, 929-935
Dodic *et al* 1998 – Clinical Science (1998) 94, 149-155

A14

The effect of periconceptional undernutrition on sensitivity of maternal glucose and fat metabolism to insulin in pregnant ewes.

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Background: Periconceptional undernutrition alters fetal growth, metabolism and endocrine status in late gestation. A possible mechanism underlying these effects could be nutritionally induced perturbation of physiological adaptation to pregnancy, which includes the development of insulin resistance.
Aim: To determine the effect of periconceptional undernutrition on sensitivity to insulin of glucose and fat metabolism in periconceptionally undernourished singleton bearing ewes in mid and late gestation.
Methods: Ewes were either well nourished (N; fed to maintain body weight ±5%) or undernourished from 61d before to 30d after mating (UN-60-30; fed to achieve and maintain 15% weight loss). Singleton bearing ewes underwent hyper-insulinaemic-euglycaemic clamp at 65 and 120d of gestation. Baseline and steady state (SS) concentrations of glucose, insulin and free fatty acids (FFA) were measured. Insulin sensitivity was calculated as the ratio of SS glucose infusion rate to SS plasma insulin concentration. Suppression of lipolysis by insulin was calculated as the percentage change in FFA concentration per SS insulin concentration. Groups were compared using factorial ANOVA with Fisher's post-hoc correction for multiple comparisons.
Results: Pregnant N but not UN ewes had lower baseline plasma insulin levels and insulin sensitivity of glucose metabolism at 65d gestation than non-pregnant ewes. Insulin sensitivity was lower in N than UN ewes for glucose metabolism at 65d and for fat metabolism at 65 and 120d.

		Baseline blood glucose $mmol.l^{-1}$	Baseline plasma insulin $\mu U.ml^{-1}$	Insulin sensitivity $mg.l.\mu U^{-1}.kg^{-1}.min$	Baseline plasma FFA $mmol.l^{-1}$	Lipolysis suppression $\%\Delta FF$ per $mU.ml^{-1}$ insulin
N	65d preg n=12	2.4±0.1	132±15	2.4±0.3	0.7±0.1	0.07±0.007
	non-preg n=6	2.5±0.1	369±116 *	3.9±0.4 *	0.7±0.2	0.10±0.006
	120 d preg n=10	2.0±0.1	230±2.7	4.9±0.6	0.7±0.1	0.14±0.020
UN	65d preg n=11	2.4±0.1	140±19	4.9±0.4†	0.5±0.1	0.12±0.010†
	non-preg n=4	2.7±0.2	184±25	5.0±0.8	0.5±0.1	0.11±0.005
	120d preg n=11	2.1±0.1	215±22	5.7±0.6	0.6±0.1	0.19±0.010†

Values are mean±SE
*p<0.05 for comparison between pregnant (preg) and non-pregnant (non-preg) animals in the same nutritional group
† p<0.05 for comparison between N and UN pregnant animals at the same gestational age

Conclusions: Periconceptional undernutrition inhibited the normal development of maternal insulin resistance in pregnancy. Impaired maternal physiological adaptation to pregnancy may be one mechanism by which periconceptional undernutrition alters growth and development of the offspring.

A15

Effect of prone sleeping on blood pressure variability in term infants

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Background: The prone sleeping position is a major risk factor for Sudden Infant Death Syndrome (SIDS). An uncompensated hypotension may play a vital role during this fatal event, however to date there is no description of blood pressure (BP) control during sleep in infants.

Aims: To determine the effect of sleeping position and sleep state on blood pressure variability (BPV), BP and heart rate (HR) as an index of BP control during sleep in infants at 2-3 mo of age, when the risk of SIDS is greatest.

Methods: Polysomnography was performed on 20 term infants born at 10-11 wks 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 and prone positions. BP was recorded 1 or 2 minute epochs with a 2 minute rest period between each measurement and this was repeated 4 times in each sleep state and each sleep position. Data were analysed beat-beat for mean arterial pressure (MAP), and heart rate HR. The standard deviation (SD) of MAP was calculated for each epoch of BP and used as an index of BPV.

Results: Results are presented in the table below. One infant was excluded due to an incomplete data set. In the prone versus supine position BPV was significantly reduced in QS; however there was no difference in AS. In AS compared to QS, BPV was higher in both positions. There was no effect of position on MAP; however MAP was higher in AS compared to QS in both sleeping positions. HR was higher in both sleep states in the prone position compared to supine, and HR was only higher in AS compared to QS in the prone.

Table: BPV, MAP and HR in each sleep state and sleep position.

	MAP BPV (mmHg)	MAP (mmHg)	HR (bpm)
QS-Supine	2.3 ± 0 †	67±2	128 ± 2
AS-Supine	2.7 ± 0	75±4†	129 ± 2
QS-Prone	1.9 ± 0 *†	66±2	131 ± 2*
AS-Prone	2.5 ± 0	71±2†	135 ± 2*†

*p <0.05, position; †p < 0.05, sleep state

Conclusions: Sleep state affects both arterial pressure and its variability in full term infants, with both BPV and MAP being higher in AS compared to QS, regardless of sleeping position. Autonomic compensations appear to be most pronounced in prone AS, as heart rate reaches its highest level in this condition in combination with the high arterial pressure and arterial pressure variability. Thus infants sleeping prone in AS may be at risk for autonomic instability and potentially for SIDS.

A16

Effects of haemorrhage in lambs born to subtotally nephrectomised mothers.

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Background: Our previous findings have suggested that maternal subtotal nephrectomy (STNx), prior to pregnancy causes fetal volume expansion. That is, fetuses of STNx mothers have high urine flow rates, low haematocrits and suppressed plasma renin levels[1]. In addition, their renin response to a haemorrhage of 20% of estimated blood volume over 20 min was delayed and attenuated[2].

Aim: To determine if the response to haemorrhage remains altered after birth in lambs born to STNx mothers.

Methods: Ewes underwent subtotal nephrectomy, which involved removal of one kidney and partial infarction of the remaining kidney, at least 2 months prior to mating. At 3-7 days of age, lambs from STNx mothers (STNxL, n=10) and control mothers (ConL, n=6) underwent surgery. Under general anaesthesia, catheters were placed in the femoral artery and veins, as well as the bladder. At ~26-27 days of age conscious lambs were subjected to haemorrhage (H) by removal of 16 ml blood/kg over 10 min. Cardiovascular responses and plasma renin levels were measured before, during and for 1h after H. At 24h post H a further blood sample was collected and post mortem was carried out. Plasma and renal renin levels were measured as the generation of angiotensin I in samples incubated with nephrectomised sheep plasma (a source of angiotensinogen) at 37ºC and pH 7.5. Angiotensin I levels were measured by radioimmunoassy.

Results: In the baseline period mean arterial pressure (MAP) was higher (STNxL 79.4±1.2 v ConL 73.7±2.2 mmHg; $p<0.01$), and plasma renin levels were lower ($p<0.05$) in the STNxL than in ConL; haematocrit was not different. In response to H, MAP decreased in the STNxL ($p<0.001$) but only tended to decrease in the ConL, hence there was a difference between the groups ($p<0.016$). During and immediately after H, plasma renin levels increased in both groups but the rise by the end of H was greater in the ConL by about 50% ($p<0.005$). At 24h, plasma renin levels were restored to normal in both groups. Kidney renin levels were similar in the two groups.

Conclusions: Subtotal nephrectomy at 3-4 weeks of age retains the attenuated renin response to haemorrhage observed in fetuses of STNx mothers. This suppression of renin release probably explains the large decrease in MAP seen in the STNxL, which is not seen in the ConL. Since by 3-4 weeks after birth the STNx lambs would have rid themselves of the excess fluid they accumulated *in utero*, their attenuated renin response at this age cannot be simply attributed to volume expansion. We now intend to determine if the reduced response persists into young adulthood and the mechanism that underlies it.

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2. Gibson *et al* (2004). PSANZ 8th Annual Conference, A21.

A17

The effect of mid-pregnancy shearing on lamb birth weights

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Introduction: Shearing ewes during early to mid pregnancy (days 30-100) has consistently increased lamb birth weight under both indoor and pastoral based production systems by up to 20% (Dyrmundsson, 1991; Kenyon *et al.*, 2003). The increase in lamb birth weights is not due to an increase in ewe feed intake (Kenyon *et al.*, 2002a; Revell *et al.*, 2002), increased gestation length (Kenyon *et al.*, 2002b) or changes in maternal thyroid hormone concentrations (Kenyon *et al.*, 2005). Shearing is a major stressor for sheep and other stressors are known to increase birth weights, including isolation of sheep (Roussel et al., 2004) and transportation of cattle (Lay et al., 1997). However, the specific aspects of shearing which cause the birth weight increase have not been identified. Therefore the aim of this study was to identify which aspect of mid-pregnancy shearing causes the increase in lamb birth weight.

Methods: The effects of the different aspects associated with mid-pregnancy shearing (between days 70 and 80 of pregnancy) on lamb birth weight were compared with mid-pregnancy shearing. The aspects of shearing that were investigated included: fasting and yarding, crutching (removal of wool from belly an around the tail), simulated shearing (all the events of shearing without removing wool), repeated isolation and repeated simulated shearing. The effect of repeated hydrocortisone injections was also investigated.

Results: Mid-pregnancy shearing of ewes increased lamb birth weights ($P<0.05$) compared with unshorn controls. However, all of the other factors that were investigated failed to increase lamb birth weights. Interestingly repeated challenges with hydrocortisone resulted in significantly ($P<0.05$) lower lamb birth weights compared to lambs born to unshorn control ewes.

Conclusions: The specific aspect of shearing that produces the increase in lamb birth weight remains unknown. However, we can conclude from these studies that neither one-off nor repeated stressors themselves are the cause of the birth weight increase. Further research may need to focus on the role of cold stress on lamb birthweights.

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A18

The effects of the extremes of maternal size within a genotype and maternal nutrition on the resulting offspring

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Introduction: A number of sheep studies have examined the effects of maternal feeding on; fetal growth, organ development, birth size, growth to weaning, or future performance of the offspring but very few have examined all of the pre-mentioned factors, let alone intergenerational effects. In addition comparisons between studies are often difficult as they are confounded by timing, length and level of the nutritional insult examined and ewe size/condition. The aim of the present large scale study was to circumvent some of these issues by examining the effects of two levels of maternal nutrition from day 21 of pregnancy in both 'light' and 'heavy' ewes.

Methods: In 2005, 400 'heavy' ewes (64.14 ±0.27 kg) and 400 'light' ewes (46.80 ±0.26 kg) selected from the extremes of a 3500 ewe commercial flock, were either offered adlib pastoral feeding conditions ('adlib') or a level at which total maternal weight increased at a level equal to the expected conceptus mass at term ('maintenance'). At 65, 100 and 140 days of pregnancy 5 twin-bearing ewes were euthanased from each treatment group and maternal and fetal measurements taken. In addition, at day 140 of pregnancy, 10 singleton bearing ewes from all groups were euthanased. A number of fetal organs were also collected for histology and gene expression analysis.

Results: At term, 'heavy-ad lib', 'light-ad lib', 'heavy-maintenance' and 'light-maintenance' ewes weighed 85.86 ±0.52, 70.88 ±0.53, 70.94 ±0.50, 58.13 ±0.51 kg respectively. There was a birth-rank by feeding interaction on lamb birth weight such that feeding regimen had no effect on singleton birth weights (5.98 ±0.09 vs. 5.93 ±0.10 kg for lambs born to 'adlib' and 'maintenance' fed ewes respectively, but twin-lambs born to 'adlib' ewes were significantly ($P<0.05$) heavier than those born to 'maintenance' ewes (5.11 ±0.07 vs. 4.62 ±0.07 kg respectively). Lambs born to the 'heavier' ewes tended ($P=0.09$) to be heavier at birth than those born to the 'light' ewes (5.49 ±0.05 vs. 5.34 ±0.06 kg respectively). At 10 weeks of age, there were no interactions between birth-rank, ewe size and feeding treatment. Lambs born to 'heavy' ewes were significantly ($P<0.05$) heavier than those born to 'light' ewes (28.82 ±0.29 vs. 27.25 ±0.31 kg respectively). Lambs born to 'adlib' fed ewes were significantly heavier than those born to 'maintenance' ewes (28.88 ±0.31 vs. 27.20 ±0.30 kg respectively).

Future directions: In early 2006, male lambs were slaughtered and live weight, carcass weight, and fat depth measurements taken. In addition the hind leg was collected for composition and meat quality analysis. Onset of oestrus will be monitored in the ewe lambs during the autumn to early winter period of 2006. In October of 2006 a cohort of single- and twin-born ewe lambs from each treatment group will be subjected to metabolic challenges. In the autumn of 2007 the ewe progeny will be bred. Blood samples will be collected during pregnancy and measurements taken from the resulting lambs at birth. In addition a cohort of ewes will be milked.

A19

Renal and cardiovascular responses in adult sheep exposed to prenatal glucocorticoid treatment

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Introduction: Sheep treated with excess glucocorticoid early in gestation show an altered renal development and gene expression in late gestation and develop high blood pressure as adults. Previous studies in adult normotensive sheep have shown that acute cortisol infusion directly increases renal blood flow (1). In this study we aimed to determine if prenatal cortisol (F) treatment alters the renal and cardiovascular response to acute F infusion in adulthood.

Methods: Adult female Merino sheep were used in all studies, previously exposed to either two days of vehicle (normal saline (NS) 12 mL/h- S group) or F (5 mg/h iv – F group) infusion, from 26 days of gestation. On the experimental day, F (5 mg/h, iv) or vehicle (normal saline, 12 mL/h, iv) infusion commenced for 6 hours after a one hour control period. Renal function was assessed by measurement of glomerular filtration rate (GFR - ^{51}Cr-EDTA) and renal blood flow (RBF - with para amino hippuric acid (pAH)). Blood and/or urine samples were obtained for determination of excretion rates, osmolality, pAH and F plasma levels. Mean arterial pressure (MAP) and heart rate (HR) were also recorded.

Results: 1. The F group tended to have higher basal plasma F concentrations compared to S group (173±25 nM vs. 84±35 nM respectively, P=0.062) accompanied by a higher RBF, but no significant increase in GFR, suggesting a deficiency in filtration efficiency. They also had consistently higher plasma glucose levels whereas other plasma electrolytes levels were not significantly different. MAP was significantly higher in F group (90±2 mmHg) compared to in S treated group (85±2 mmHg, P<0.05) whereas there was no difference in HR. **2.** During acute F infusion when equivalent plasma F concentrations were achieved in both groups of animals (S group = 340±26 nM vs F group = 336±32 nM, P=NS), F infusion caused an increase in RBF in both groups which was significantly greater in F group compared to S group (436±118 vs 263±59 mL/min, respectively, P<0.05). **3.** The increase in GFR was significantly increased in both groups in response to acute F infusion (F group +9±3 vs S group +12±4 mL/min, P<0.05 compared to vehicle) but the increase was similar in both groups, again suggesting a dissociation between RBF and GFR in F treated animals. **4.** The changes in renal function in response to acute F infusion occurred without significant changes in MAP or HR. **5.** Acute F infusion had no significant effect on electrolyte (Na, K, Cl) excretion in S or F groups when compared to vehicle infusion.

Conclusion*:* Even though prenatal exposure to F has been shown to lead to a significant deficit in nephron endowment (2) in sheep, they can still maintain a normal GFR (implying an increased single nephron FR). Previous studies have shown that the increase in RBF in response to acute F infusion involves the renal nitric oxide/prostaglandin systems (1) implying that these systems are altered in sheep exposed to early prenatal F.

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A20

Does IUGR due to twinning affect postnatal arterial pressure?

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Background: In humans, twinning causes IUGR, primarily during the third trimester, resulting in a reduction in birth weight of 0.5-1.0 kg. Twins have been studied extensively in an attempt to separate genetic from environmental influences in the development of later diseases. Some studies in humans have found that twins are at increased risk of elevated blood pressure after birth while others find the converse or no effect. A recent study in sheep has shown that natural twinning (~30% reduction in birth weight) followed by twin nursing leads to further growth restriction, hypernatremia, reduced GFR and elevated arterial pressure at 3 weeks after birth (Ross et al, 2005). Owing to uncertainties about the long-term effects of twinning we have studied lambs from twin pregnancies up to 9 weeks after birth.

Methods: We compared data from 8 singleton lambs with data from 5 pairs of twins, all born vaginally at term and reared with their mothers. Lambs were weighed at birth and then at regular intervals to 9 weeks. At 9 weeks after birth, arterial pressure and heart rate were recorded via arterial catheters on 2 consecutive days while the animals rested in slings. Blood samples were collected prior to autopsy.

Results: At birth, twins were 26% lighter than singletons (3.4±0.2 vs 4.6±0.2 kg, $p<0.05$). After birth twins remained lighter than singletons, and at 9 weeks were 25% lighter than singletons (13.4±0.5 vs 17.9±1.0 kg). At 9 weeks, mean arterial pressure (MAP) tended to be lower in twins than singles (69±5 vs 83±6 mmHg; $p=0.09$). Systolic pressure was significantly lower in twins than in singles (83±5 vs 109±6 mmHg, $p<0.05$), whereas diastolic pressure was not different (twins, 60±6 vs singles 69±6; mmHg, $p=0.31$). Heart rate tended to be lower in twins than singles (86±7 vs 106±8 bpm; $p=0.08$). Systolic pressure was significantly correlated with current body weight ($p=0.048$). There was no evidence of differences in plasma sodium concentration or osmolality between groups; however, twins had lower blood glucose and higher urea levels than single lambs.

Conclusions: Ovine twins in which postnatal body weight increases proportionate to that of singletons do not exhibit hypertension or hypernatremia at 9 weeks of age. Differences between the present study and that of Ross et al (2005) may be due to differences in postnatal nutrition. Our data are consistent with two previous ovine studies in which IUGR resulted in lower rather than higher MAP after birth: IUGR induced by late gestational placental embolization led to a reduction in MAP of 4 mmHg (Louey et al, 2000); IUGR induced by repeated maternal antenatal glucocorticoid exposure led to a 10 mmHg reduction in MAP (Moss et al, 2001). We conclude that the effects of twinning on postnatal arterial pressure may be due to combined influence of both prenatal and postnatal nutrition.

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A21

Prenatal immune activation and type of stress exposure: Differential effects on stress induced analgesia.

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Background: The induction of a state of analgesia is part of a set of coordinated, physiological, adaptive responses following stress exposure. A down-regulation of pain perception at the time of stress exposure means that cognitive function and energy can be focused on those systems required for dealing immediately with the stressor. In the past, research has indicated that early life events can pre-determine the set points of the stress system, with particular emphasis on the ontogeny of the hypothalamic-pituitary-adrenal (HPA) axis. Our laboratory has shown previously that prenatal immune activation in the rat, results in offspring with a hypersensitive HPA axis, as indicated by increased corticosterone levels following stress exposure. This study aims to identify whether this hypersensitive stress system, caused by maternal immune activation during pregnancy, has implications for the level of analgesia induced following stress exposure.

Methods: Immune activation during pregnancy occurred following exposure of pregnant *Fischer 344* dams to bacterial endotoxin (*Salmonella enteritidis,* 200ug/kg, s.c.) on gestational days 16, 18 and 20 while control dams received an equivolume of saline. In adulthood, both male and female offspring were subjected to one of two stress paradigms - restraint or a forced cold-water swim. The restraint stress groups were placed in a standard Plexiglas restraint tube for a 30-minute period, with the control groups receiving an equal time of food and water deprivation in their home cages. Changes in pain sensitivity were assessed using the tail immersion test, where the latency to remove the tip of the tail from hot water (54oC) is measured. Pain was assessed prior to restraint, halfway through restraint, 0 and 60 minutes following the restraint period. The second stress group was exposed to a three-minute swim in cold water (15oC). Following removal, animals were dried and placed in a towel-lined box until the first assessment of pain. Prior to, 5 and 30 minutes following removal from the water, pain thresholds were assessed using the tail immersion test. Control offspring were placed in the towel-lined box between the baseline and five minutes pain assessment.

Results: While a significant analgesic response was induced in the control offspring during the exposure to restraint ($p<.05$), this analgesic response was exaggerated in the offspring of endotoxin treated mothers, with a significant increase in tail withdrawal latency compared to the control offspring ($p<.05$). This stress induced analgesic response was only evident during the stress exposure in both the prenatal treatment groups, with a return to baseline levels upon removal from restraint. As expected, no analgesic response was observed in the non-restraint, food and water deprived groups. The cold water swim also induced a significant analgesic response in the control offspring that was evident at 5 minutes ($p<.05$). The offspring of endotoxin treated dams, however, showed no evidence of a stress induced analgesic response to this stressor, with tail withdrawal latencies remaining at baseline levels throughout the testing period.

Conclusions: This study is the first to show that activation of the maternal immune system during pregnancy differentially affects the level of analgesia induced by stress in the adult offspring. Further, this study has shown that the production of stress-induced analgesia in offspring of endotoxin treated mothers was dependant on the stressor used, as only the restraint stress produced analgesia in this group, while both the cold water and restraint stress produced analgesia in the control offspring.

A22

Comparison of effects of mild hyperthermia or endotoxin on hypoxia-induced neural cell death in chick embryos in ovo

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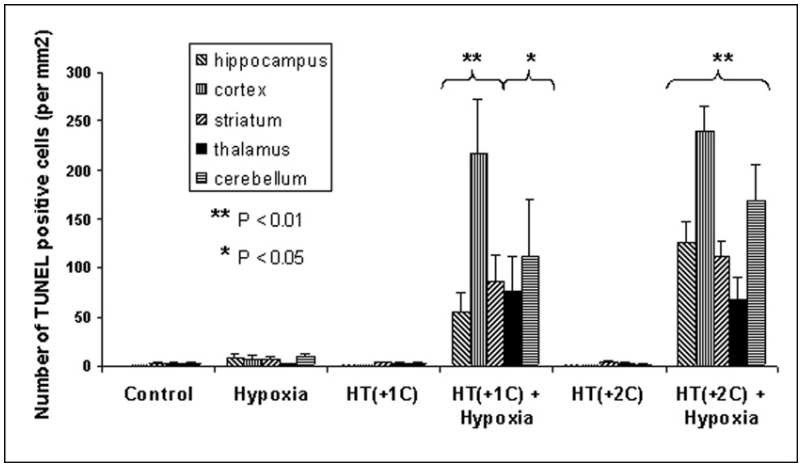
Introduction: Intrapartum maternal pyrexia is associated with an increased incidence of adverse neonatal neurological outcome; this may be because pyrexia is a marker of infection or because hyperthermia exacerbates hypoxia related brain damage. The aim of this study was to compare the effects of mild hyperthermia with endotoxin pretreatment on neural cell death following global hypoxia in chick embryos in ovo.

Methods: The following groups of Leghorn chick embryos were studied on incubation day 19 (chicks hatch at day 21) a) hypoxia alone (FiO2=4%) for 1hr b,c) hyperthermia alone at 1°C or 2°C above normal incubation temperature for 7hrs d,e) combination of both hypoxia and hyperthermia, with hypoxia commencing 4 hours after the increase in temperature or f) hypoxia combined with 3mg Salmonella Typhii LPS, administered 4 hours pre-hypoxia. Brains were extracted 24 hours post hypoxia for TUNEL labeling. Brain damage was quantified by comparing TUNEL positive cells in various brain regions, including striatum, thalamus, hippocampus, cerebellum and cerebral cortex. Statistic analysis was carried out using one-way ANOVA.

Results: Hyperthermia (both 1°C or 2°C) and hypoxia (ambient oxygen 4%) alone did not lead to significant brain injury in any area. By contrast, the combination of hypoxia and a mild rise of incubation temperature (1°C) lead to wide spread significant brain damage, with the cortex, hippocampus and striatum more badly hit ($p<0.01$) than cerebellum and thalamus ($p<0.05$) (see figure). A further increase in temperature to 2°C above normal levels, combined with hypoxia, caused even more severe loss of neural cells, especially in the hippocampus and cerebral cortex. Comparing the hyperthermia/hypoxia groups (d,e) with LPS/hypoxia (f), a different pattern of cell loss was observed, with similar degrees of damage in hippocampus but significantly more apoptotic cells observed in cortex, striatum and cerebellum following hyperthermia than LPS ($p<0.001$).



Conclusion: In this model neither global hypoxia or hyperthermia led to neural cell death. However, even a mild hyperthermic challenge (1°C) dramatically increased cell death following hypoxia. When compared with LPS pre-treatment, cell death was more severe, and widespread after hyperthermia. These data suggest that pyrexia does not need to be infection related to lower the threshold at which hypoxia causes neural cell death.

A23

Neuropharmacology of behaviour in the mid-gestation fetus

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Introduction: All developing vertebrates studied to date exhibit a form of motor activity in which all the musculature of the developing organism is active for a period of time followed by a period of inactivity. This form of behaviour, known as cyclic activity, is not appropriate for postnatal life where purposive behaviours, such as feeding and grooming, require precisely timed and independent activation of different muscles.

As an initial stage in the development of independent activation of muscles, cyclic activity ceases at approximately G65 in the fetal sheep, but the precise mechanisms behind the loss of cyclic activity are largely unknown. However, it is known that spinally projecting axons from the brainstem are involved in the process. The aim of this study is to determine the actions of specific neurotransmitters in the mid-gestation fetal sheep with the specific aim of determining what neurotransmitters are responsible for the loss of cyclic activity at this time.

Method: Using the fetal sheep as a model, recordings were made from the diaphragm, external oblique and vastus lateralis muscles between G78-G120. Recordings were made before and during the infusion of specific agonist and antagonists into the CSF via a catheter inserted into the sub-arachnoid space.

Results: Results show that glutamate is the major excitatory neurotransmitter, while GABA and glycine are the major inhibitory neurotransmitters in the spinal cord of the mid-gestation fetal sheep. Noradrenaline and dopamine did not have a potent action on the behaviour generated in the fetal spinal cord. On the basis of results from 3 fetuses, methysergide (a serotonin antagonist) infusion in fetal sheep, after the transition in motor activity has already occurred, results in a reversion of behaviour to cyclic activity.

Conclusion: These results suggest that serotonin is involved in the transition of motor activity in the fetal sheep, and therefore likely to be involved in the transition from cyclic to independent muscle activation in other vertebrates, including the human.

A24

The role of the sympathetic nervous system in the cerebral circulation during hypercapnia in sleep

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Introduction: The cerebral vessels are sensitive to fluctuations in arterial blood gases, particularly carbon dioxide. Studies have shown that hypercapnia results in decreased cerebrovascular resistance (CVR) and increase in cerebral blood flow (CBF) (1) Furthermore, studies have shown the increase in CBF during REM sleep is augmented by CO_2 (2) and sympathectomy (3). However, the potential role of the cerebral sympathetic innervation on hypercapnic responses in sleep is unknown, and may be of importance in limiting large CBF increases in such conditions as sleep apnea. In this study we hypothesised that sympathetic innervation to the cerebral circulation attenuates cerebral blood flow increases that accompany increases in $PaCO_2$ and REM sleep.

Methods: In pilot studies, lambs (n = 2, 2-4 weeks PA) were instrumented under halothane (2% halothane, 50%oxygen, balance NO_2) general anaesthesia to record cerebral blood flow (CBF), for blood sampling, and sleep staging (ECoG, EMG & EOG). A facemask placed on the lamb's head was used for manipulation of gas concentrations. The lamb was subjected to three 60 seconds tests of hypercapnia (8%) during wakefulness (QW), quiet sleep (QS) and active sleep (AS) before and after superior cervical ganglionectomy.

Results: Confirming previous studies, baseline CBF was greater in the sympathectomised lamb. CBF also varied with sleep stage, AS>QW>QS, in both the intact and sympathectomised group. In response to hypercapnia CBF increased as follows: Control, QW (12 ± 3 %, mean ± SD), QS (14 ± 5 %), AS (32 ± 13 %) from baseline. Sympathectomised, QW (19 ± 6 %), QS (15 ± 5 %), AS (21 ± 9 %).

Conclusion: Our data confirm previous findings that superior cervical ganglionectomy results in increased baseline cerebral blood flow and that CBF varies between sleep states, being greatest in AS. Preliminary data show no difference in the CBF response to hypercapnia between groups, although the flow increases were greatest in AS (perhaps due to greater cardiovascular sensitivity to arterial CO_2) but no limiting effect of the autonomic nervous system.

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A25

Endotoxin effects on cerebral circulation in fetal lambs

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Introduction: Intrauterine infection increases the risk of long term brain injury in surviving infants, and much effort has been devoted to explore the mechanism(s) of brain injury. Lipopolysaccharide (LPS) induced disruption of the fetal cerebral circulation, particularly of cerebral blood flow (CBF), is a likely mechanism but this is unclear. It has been reported that, using microspheres, there was no alteration in global CBF in fetal lambs after endotoxin exposure (Dalitz et al, 2003), but in another study using the same method, LPS depressed CBF (Garnier et al, 2001). In this study we aimed to clarify the uncertainty by employing a continuous CBF measurement method to assess whether the CBF response to LPS is biphasic.

Aim: To establish the exact time course of changes in the fetal cerebral circulation after LPS induced inflammatory insult by continuous measurement.

Methods: Pregnant ewes (n = 4) of gestational age 120-128d were anaesthetized and the fetuses were instrumented for recording cerebral blood flow (CBF, Transonic™ flow probe on the superior saggital sinus), carotid arterial pressure (Pca), intra-cranial pressure (Pic), and amniotic fluid pressure (Paf). A jugular venous (JV) catheter was implanted for drug administration. Cerebral vascular resistance (CVR) was calculated as (Pca-Pic-Paf)/CBF. Studies began after 48 hour postoperative recovery with a control study, then LPS (1μg/kg) infusion via JV.

Results: Three fetuses survived and one aborted following one dose of LPS. In the survival group, blood pressure was unchanged at 2 hour and fell by 25±9 % (mean±SE, $P<0.05$, ANOVA) at 4 hours after LPS infusion. CBF rose by 52±8% at 2 hour and increased to 70±16% (mean±SE, $P<0.05$) at 4 hours post LPS infusion, and remained elevated for 12 hours. CVR increased at 2 hour by 24±17%, then fell by -20±4% (mean±SE, $P<0.05$) at 4 hour of infusion, and remained low thereafter. Arterial PO_2 was reduced from 23±1 to 20±1 mmHg at 2 hour post infusion, and remained depressed by -5 mmHg in the subsequent 12 hours. In the aborted animal, blood pressure and CBF were maintained at control levels until 2 hours prior to death, when they began to decline.

Conclusions: Endotoxin has significant acute impacts on the fetal cerebral circulation. CVR is first increased, and then decreased, while CBF remains elevated against a background of persistent hypoxaemia.

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A26

Effect of perinatal asphyxia on survival and postnatal behaviour in Spiny Mouse with and without maternal creatine dietary supplementation

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Background: Perinatal Asphyxia is a known cause of morbidity and mortality in newborn human infants. Asphyxia results in a biochemical cascade is in which energy failure, excitoxicity and oxidative stress leads to brain damage and subsequently to motor impairment, mental retardation, seizures and cerebral palsy. There is evidence to suggest that creatine may offer protection by providing an energy buffer during prolonged periods of severe hypoxia, ischaemia, or both. The overall objective of this project was to assess the capacity for creatine to protect the fetus from asphyxia induced at birth in an animal model where brain development is similar to the human infant at term.
The biological and neurobehavioral development of the precocial spiny mouse (*Acomys cahirinus*) at term renders it a species with brain development comparable to the human infant. In this study we developed a model of perinatal asphyxia at term, and then examined the effects on behaviour. It was predicted that asphyxiated animals would show neurological deficits when compared to control animals and this deficit would be prevented or reduced in animals where the maternal diet had been supplemented with creatine from mid-gestation.

Methods: Pregnant dams were housed under normal housing condition with the experimental groups fed either normal rat chow, or a rat chow supplemented with 5% creatine monohydrate from day 20 of gestation (term is 40 days). Intrauterine global asphyxia was induced on day 39 by excising the uterus from the dam after cervical dislocation, and immersing it in a saline bath at 36-38 °C. After 7.5-8 mins the fetuses were removed from the uterus, the mouth checked to ensure an open airway, and the animals allowed to self-resuscitate with some tactile stimulation of the skin, if necessary. The survivors were then cross fostered to dams that had spontaneously delivered their own pups within the preceding 24 h. Behavioural testing of the pups was then assessed between postnatal day1-15 using - accelerated Rotarod test; footprint assessment of gait; and exploration in an open field test. Other pups from each treatment group were killed immediately after removal from the uterus for enzymatic analysis of ATP, phosphocreatine (PCr), creatine and total creatine concentrations of the brain and heart at the time of birth.

Results: 67% of the fetuses from control fed dams survived birth asphyxia compared to a 100% survival rate for fetuses from creatine supplemented dams. Fetuses exposed to asphyxia had significantly reduced ATP and PCr concentrations ($p<.001$) and increased creatine concentrations ($p<.01$) compared to non-asphyxiated fetuses delivered by caesarean section, but there was no difference between fetuses from control and creatine supplemented dams. Behavioural assessment showed that birth asphyxia resulted in neonates traveling significantly shorter distances ($p<.05$) and jumping less ($p<.05$) in the open field test; walking with significantly increased stride length ($p<.05$) in the footprint test; and having an increased ability to remain on the rotating drum in the Rotarod test ($p<.05$) - on postnatal days 1-2 compared to control animals.

Conclusion: The current study developed a new and potentially useful model of birth asphyxia that induced a subtle but persistent postnatal neurological deficit. Although the maternal creatine-supplemented diet did not prevent neurological deficits it did increase the capacity of fetuses to survive the asphyxial insult. We speculate that this is due to either prolonged maintenance of cardiac function or reduced oxidative stress in the brain during the asphyxia.

A27

Measuring the impact of prenatal stress on hippocampal function in a new small animal model

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Background: It is widely recognised that many long term and intractable physical and behavioural problems in children have origins in utero. Perinatal brain injury is often caused by cerebral hypoxia/ischaemia, cerebral haemorrhage, or ascending intrauterine infection, and invariably includes degrees of damage to the hippocampus. The hippocampus is a highly plastic and vulnerable brain region, and a crucial centre for memory and spatial learning in the mammalian brain. Long term potentiation (LTP) - a persisting strengthening of synaptic transmission between neurons, and thought to be the molecular mechanism of learning and memory - occurs in the hippocampus between neuronal populations known to be affected by chronic stress, including hypoxia and glucocorticoid exposure.

Aim: To establish a model of prenatal hypoxic/ischaemic stress in the late gestation Spiny Mouse and identify the structural and functional impact on hippocampal development and on postnatal indices of learning and memory.

Animal Model: The Spiny Mouse (*Acomys cahirinus)* is a precocial murid species that has several unusual neurophysiological characteristics, most notably a relatively thin cerebral cortex and large hippocampus. Spiny mice are particularly suitable for use in a prenatal brain injury model as they display a large number of spontaneous motor activities and reflexes that can be readily tested from birth (due to the more advanced development of the brain compared with conventional [altricial] rodents), and the longer gestation (40 days) allows experimental insults to be applied during pregnancy.

Methods: Using freshly prepared 300 µm thick coronal slices of brain, LTP is evoked by high frequency stimulation (HFS) of axons in the Schaffer Collateral Pathway, while recording excitatory post-synaptic potentials (EPSPs) from CA1 pyramidal neurons. To date, this has been done successfully in immature postnatal Spiny Mouse at 20 days of age, and currently we are determining development of LTP from 35 days gestation to 40 days after birth. We have been able to measure LTP as an increase in the amplitude of EPSPs after HFS of up to 170% compared to pre-HFS control, and remaining at >140% for more than 100mins. We will now determine the effects of various prenatal stresses on LTP in the postnatal brain. Models of prenatal stress will include (i), uterine artery occlusion for 20mins at 30 days gestation with natural delivery at term; (ii), 7-8 min birth asphyxia at term; (iii), exposure to excess glucocorticoid (dexamethasone) at 20-22 days gestation with delivery at term; (iv), exposure to maternal endotoxin [LPS] at 30-35 days. Postnatal behavioural testing includes the Open Field estimation of exploration, motor co-ordination using the Rotarod, and testing of memory and learning by passive avoidance testing and the Morris water maze.

A28

Protein 14-3-3 in the CSF of newborn rats following hypoxia-ischemia

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Background: A hypoxic-ischaemic (HI) insult is one of the major causes of morbidity and mortality in term infants. To initiate neuroprotective interventions, early indicators of brain damage are required. The protein 14-3-3 family are a group of highly conserved proteins ubiquitously expressed in a wide range of organisms. Of the seven isoforms, only the γ isoform is highly specific to the brain. In spite of reports in adult animals and humans, protein 14-3-3 has never been measured following a hypoxic-ischaemic insult in a newborn animal experiment.

Aims: To measure protein 14-3-3 in the CSF of newborn rats following a HI insult

Methods: 10-day-old rats were exposed to hypoxia-ischaemia using the Levine model. CSF was collected at 1, 2, 4, 6, 12, 24, and 72 hours after the insult. A control group was subjected to sham surgery but not to the HI insult. Protein 14-3-3γ was quantified by Western blotting. The optical density of bands was determined using Image PC software (Scion). The rats were sacrificed after collection of the CSF and the brains taken for histological examination.

Results: In the control group, protein 14-3-3γ did not increase significantly over time. In the study group, there was a significant increase in protein14-3-3γ as early as 2 hours after the end of the insult, peaking at 4 to 6 hours and returning to basal levels by 72 hours.

Conclusions: Protein 14-3-3γ increases in the CSF of newborn rats following a HI insult. The presence of protein 14-3-3 in CSF is a good indicator for neuronal loss or damage. Before protein 14-3-3- may be used to guide clinicians to accurately predict outcome and time of an insult to potentially initiate neural rescue therapies, these results have to be verified in humans and protein 14-3-3 measured in blood.

A29

Cyclical response of newborn piglets to global hypoxia

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Background*:* The newborn piglet responds to global hypoxia by increasing mean arterial blood pressure and heart rate, and increasing peripheral vasoconstriction in order to preferentially deliver oxygen to the brain, heart and adrenals. However if hypoxia continues these compensatory mechanisms begin to fail and cerebral blood flow falls increasing the risk of cerebral damage. We have observed that, as blood pressure begins to fall, some animals exhibit a cyclical pattern of blood pressure and heart rate changes.

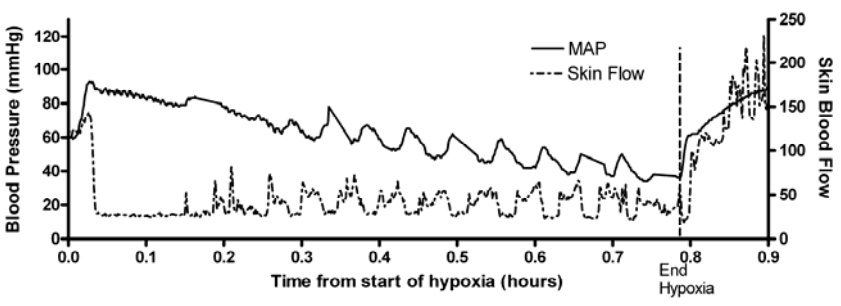
Aim*:* To characterize the cyclical changes in blood pressure observed in some newborn piglets during global hypoxia, and to determine their relationship to the severity of neural injury resulting from hypoxia.

Methods*:* Twenty anaesthetised and ventilated newborn piglets (<1 day old) were subjected to global hypercapnic hypoxia induced by reducing the FiO_2 to 10% and reducing the ventilation rate from 30 to 10 bpm. Arterial blood pressure and heart rate were monitored continuously from an umbilical artery catheter. Skin blood flow was monitored by laser Doppler flowmetry. Blood was sampled for analysis of arterial blood gases, and for catecholamine and cortisol concentrations. Six control animals were subjected to all of the above procedures except for the hypoxia. Animals were killed 6 hours after the end of hypoxia and the severity of neural injury was assessed using a combination of cerebral impedance, EEG and MAP2 immunohistochemistry. Animals were divided into good and bad outcome groups based on these measurements.

Results*:* A cyclical pattern of blood pressure and heart rate changes was observed during hypoxia in 11/20 animals (Fig 1). This cyclical pattern began on average 20.2 minutes after the beginning of hypoxia and continued until the end of hypoxia. The average period of the cycles was 3.6min and the amplitude was 21.2mmHg or 33.8bpm. The cyclical pattern was also observed in skin blood flow with changes usually occurring in the opposite direction to blood pressure changes (Fig 1). Five/11 animals which exhibited the cyclical changes were classified as having a good outcome while 2/9 animals without cyclical changes were classified as good outcome.

Conclusions*:* In response to global hypoxia some animals respond with a cyclical pattern of arterial blood pressure, heart rate and skin blood flow changes. The origin of the changes is unknown and warrants further investigation given their possible association with good outcome.

Fig 1: Arterial Blood Pressure and Skin Blood Flow During Hypoxia



A30

Antenatal exposure to inflammation reprograms postnatal airway responsiveness and immune status.

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Background: Epidemiological studies show the intrauterine environment affects the risk of developing childhood asthma. Antenatal exposure to inflammation can modulate lung and systemic immune function in the fetus, and may subsequently affect airway reactivity during postnatal life. We investigated the effects of antenatal inflammation on postnatal immune response and airway reactivity after sensitization to house dust mite.

Methods: Intra-amniotic injections of either lipoploysaccharide (LPS: *E coli* 055:B5; 10mg) or saline (control) were given to pregnant sheep at 120, 130 and 140 days of pregnancy. Ewes were allowed to deliver spontaneously at term and blood was collected from newborn lambs within 48 hours of delivery. Lambs were exposed to house dust mite allergen (HDM; 50 µg) or saline by subcutaneous injection at 1, 3 and 5 weeks of postnatal age. At 8 weeks, lambs were sedated for challenge with HDM allergen (1mg); 48 hours later, lambs were paralysed for assessment of airway reactivity to methacholine challenge (MCh; 0.01 to 10%) using low-frequency forced oscillatory mechanics. Lambs were euthanized and immune status was assessed from blood and broncho-alveolar lavage fluid (BALF) differential cell counts.

Preliminary Results: LPS-exposed lambs had fewer circulating eosinophils than control at birth (LPS, $0.05x10^9 \pm 0.02x10^9$ eosinophils/L; control $0.07x10^9 \pm 0.02x10^9$ eosinophils/L; p=0.045) and at 8wks postpartum (LPS $0.19x10^9$ +/- $0.05x10^9$ eosinophils/L; control $0.42x10^9$ +/- $0.09x10^9$ eosinophils/L; p=0.05). HDM exposure did not affect BALF or circulating differential cell counts but sensitization to HDM could not be definitively confirmed. Airway resistance and tissue damping were lower than control after prenatal LPS exposure; there was no effect of postnatal HDM exposure.

Conclusions: Our preliminary data suggest postnatal immune status and airway reactivity are modulated by exposure to intrauterine inflammation, consistent with data from humans showing a protective effect of prenatal inflammation against asthma. At this stage of our investigations, we are unable to definitively determine the relative contribution to postnatal immune function and airway reactivity of the pre- and post-natal environments.

A31

Effects of medroxyprogesterone acetate on intrauterine inflammation induced by amniotic fluid ureaplasma infection.

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Background: Recent clinical trails have shown that progesterone can reduce the rate of preterm birth in women considered to be at high risk. Preterm birth is often associated with intrauterine infection/inflammation; ureaplasmas are the microorganisms most frequently involved. Progesterone-induced suppression of intrauterine inflammatory processes may be responsible for prevention of preterm birth. We hypothesised that medroxyprogesterone acetate (MPA) would decrease fetal and intrauterine inflammation induced by amniotic fluid ureaplasma infection.

Methods: Ewes bearing single fetuses received an intra-amniotic injection of *Ureaplasma parvum* serovar 6 ($20x10^6$ colony forming units) on day 80 (n=9; control) or 85 (n=6) of pregnancy (term is 150 days). Those that received ureaplasmas at 85 days of pregnancy received a maternal intramuscular injection of MPA (150mg) on day 108. Ewes and fetuses were killed on day 123 or 124 for collection of tissues to assess fetal and intrauterine inflammation. The groups of sheep were from different experiments, conducted in separate consecutive years. Data were compared between groups using t-test or rank sum test, as appropriate.

Results: Fetal body weight at delivery was not different between control (2.7±0.2 kg) and MPA (2.5±0.1 kg) groups; lung weights also were equivalent. Infiltration of the chorioamnion by inflammatory cells was lower than control in the MPA group and amniotic fluid IL-8 concentrations were lower ($p<0.001$). Fetal total white blood cell counts were similar between groups but lymphocyte and monocyte numbers were higher ($p<0.05$) in the MPA group. Maternal total white blood cell counts were higher than control in the MPA group ($p=0.03$); lymphocyte numbers were also elevated ($p=0.02$). There were no neutrophils in bronchoalveolar lavage fluid (BALF) from fetuses exposed to MPA but there were $2.81x10^6 \pm 1.08\ x10^6$ neutrophils/kg body weight in BALF from controls ($p=0.008$). IL-8 levels in BALF from MPA-exposed fetuses were lower than control ($p=0.002$); Il-8 levels in lung tissue also were reduced ($p=0.002$). Expression of IL-8 mRNA in lung tissue from MPA-exposed fetuses was lower than control ($p=0.02$); IL-1 and IL-6 mRNA expression in lung tissue was not different between groups. Lung compliance and BALF surfactant lipid content were not different between groups.

Conclusions: Our preliminary investigation suggests that fetal and intrauterine inflammation induced by intra-amniotic ureaplasma injection in sheep can be attenuated by subsequent maternal MPA injection. Such a decrease in intrauterine inflammation may underlie the reduction in the incidence of preterm birth in women at high risk who are treated with progesterone.

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