THE TWENTY-SECOND FETAL AND NEONATAL WORKSHOP OF AUSTRALIA AND NEW ZEALAND

South Stradbroke Island Resort,

Runaway Bay, Queensland

Australia

18-19 April, 2008

2008 Organising Committee

Barbara Lingwood	University of Queensland
Rob DeMatteo	Monash University
Karen Moritz	University of Queensland
Richard Harding	Monash University

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FETAL & NEONATAL WORKSHOP 2008
PROGRAM OUTLINE

Thurs 17th April

Arrive & Registration

Friday 18th April

7:30-9:00
Breakfast (Mangroves Restaurant)

9:00-10:45
Session 1 (Tradewinds)

10:45-11:15
Morning Tea (Tradewinds)

11:15-1:00
Session 2 (Tradewinds)

1:00-4:00
Lunch (Mangroves Restaurant), Recreation
& Afternoon Tea (3:30 Tradewinds)

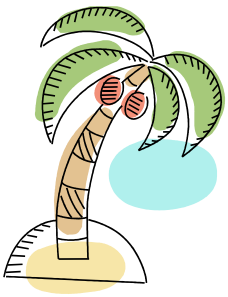
4:00-5:50
Session 3 (Tradewinds)

6:30-7:00
Pre-Dinner Drinks (Broadwater Bar Pool)

7:00 till late
Conference Dinner (Broadwater Bar Pool)

Saturday 19th April

8:00-9:30
Breakfast (Mangroves Restaurant)



9:30-11:15
Session 4 (Tradewinds)

11:15-11:45
Morning Tea (Tradewinds)

11:45-1:30
Session 5 (Tradewinds)

1:30-7:30
Lunch (Mangroves Restaurant), Recreation,
Afternoon Tea (4:00 Tradewinds) & Dinner (own arrangement)

7:30-9:15
Session 6 (Tradewinds)

Sunday 20th April

Breakfast (Mangroves Restaurant 7:30-9:00), Relaxation,
Lunch (Mangroves Restaurant 12:30) & Departure

Acknowledgments

We acknowledge the generous financial support of NGED to this workshop. We also acknowledge the contribution of Ms. Vicki Stacy, Ms. Nadine Brew, and Dr. Rosemary Horne to the organisation of this workshop.

FETAL & NEONATAL WORKSHOP SCIENTIFIC PROGRAM

Friday 18th April

Session 1 - Chair: Barbara Lingwood			
A1	9:00	Jonathan Bensley	The effect of premature birth on the number of cardiomyocytes in the postnatal lamb
A2	9:15	Eugenie Lumbers	Sexual dimorphism of cardiac myocytes in the fetal sheep and the effects of IGF-1 treatment
A3	9.30	Oksan Gezmish	Vitamin D insufficiency affects cardiomyocyte number in the left ventricle of 4 week old Sprague-Dawley rats
A4	9:45	Janna Morrison	The intrauterine growth restriction-induced delay in cardiomyocyte binucleation is related to hypoxia not hypoglycemia
A5	10:00	Kelly Kenna	An investigation into fetal arterial stiffness following maternal alcohol infusion in sheep
A6	10:15	Reetu Singh	Sex differences in the progression of cardiovascular disease in sheep following fetal unilateral nephrectomy
	10:30		*General Discussion*

10:45-11:15 MORNING TEA

Session 2 - Chair: Frank Bloomfield			
A7	11:15	Vera Golder	Assessment of autonomic cardiovascular markers as indicators of clinical compromise in ill preterm infants during the first days of life
A8	11:30	Alistair Gunn	Deep-hypothermic circulatory arrest during the arterial switch operation is associated with abnormal intracerebral oxygenation in the post-operative period
A9	11:45	Nicole Smith	Effect of premature birth on heart rate and blood pressure during sleep
A10	12:00	Takeshi Takami	Comparison of the relationship between cerebral perfusion and cardiac function in ELBW infants and VLBW infants
A11	12:15	Heidi Richardson	Swaddling infants: Effects on arousability from sleep
A12	12:30	Tamas Zakar	Glucocorticoid regulation of prostaglandin synthesis in the human fetal membranes: Duality in the style of Dr. Jekyll and Mr. Hyde?
	12:45		*General Discussion*

1:00-4:00 LUNCH & RECREATION

Session 3 - Chair: Julie Owens			
	4:00	David Walker	Prof Adrian Walker - a view through the Retrospectroscope
A13	4:20	Andrea Lee	Modulation of T cell subsets associated with intra-amniotic ureaplasma is damped by maternal progesterone and betamethasone treatments
A14	4:35	Aaron Smith	Neurosteroid profiles in fetal, maternal and neonatal guinea pigs using Gas Chromatography- Mass Spectrometry
A15	4:50	Laura Stamp	Does chorioamnionitis infection affect nephron formation in the human fetal kidney?
A16	5:05	Keiji Suzuki	Effects of intra-amniotic endotoxin on postnatal organ growth in rats
A17	5:20	Robert DeMatteo	Effects of endotoxin in fetal sheep: is erythropoietin protective?
	5:35		*General Discussion*

6:30-7:00 DRINKS

7:00 CONFERENCE DINNER

Saturday 19th April

Session 4 - Chair: Adrian Walker			
A18	9:30	Julia Pitcher	Differentiating the influence of gestation length and birthweight percentile on motor cortical development in children
A19	9:45	David Walker	Inquiry into the mechanisms of folding of the cerebral cortex during fetal development in sheep; biomechanical explanations based on MRI observations
A20	10:00	Roger Smith	Use of mathematical modelling to predict pregnancy outcomes and behaviour of the uterus
A21	10:15	Nadine Brew	Developing a novel animal model for studying the postnatal effects of neonatal chronic lung disease: preliminary observations
A22	10:30	Megan Probyn	The GenomeLab GeXP genetic analysis system: analysing multiple genes in one sample
A23	10:45	Kathy Gatford	Sustained hyperglycaemia in the sheep- a novel model to test beta cell plasticity
	11:00		*General Discussion*

11:15-11:45 MORNING TEA

Session 5 - Chair: Eugenie Lumbers			
A24	11:45	Lina Gubhaju	Pre-term birth: Effects of nephrogenesis in the human kidney
A25	12:00	Simon Hew	Cross spectral analysis and the quantification of cerebral autoregulation in preterm infants
A26	12:15	Alison Kent	Effect of indomethacin, ibuprofen and gentamicin on glomerular number in a neonatal rat model
A27	12:30	Mark Oliver	Sporidesmin-induced maternal liver damage prior to conception alters fetal organ growth and placental morphology in the late gestation sheep fetus.
A28	12:45	Kathy Gatford	Placental restriction and small size at birth in sheep; changes in pancreatic gene expression explain loss of beta-cell function and up-regulation of beta-cell mass
A29	1:00	Anne Jacquiery	Rapid postnatal growth is associated with later reduced glucose tolerance in pre-term but not term lambs
	1:15		*General Discussion*

1:30-7:30 LUNCH, RECREATION & DINNER

Session 6 - Chair: David Walker			
A30	7:30	Kitty Bach	The effect of ventilator gas flow rates on markers of acute lung injury in preterm lambs
A31	7:45	Brad Edwards	Respiratory instability: Hyperventilation and hypoxia in the lamb and human infant versus the adult
A32	8:00	Megan O'Reilly	Airway remodelling in a model of ventilator-induced injury of the very immature lung
A33	8:15	Scott Sands	Impact of postnatal increase in oxygen sensitivity on respiratory instability: Theory
A34	8:30	Carryn McLean	Akt and MAPK signaling in ventilated preterm lamb diaphragms
A35	8:45	Foula Sozo	The effects of alcohol exposure on the growth, maturation and inflammatory state of the fetal lung
	9:00		*General Discussion*

THE EFFECT OF PREMATURE BIRTH ON THE NUMBER OF CARDIOMYOCYTES IN THE POSTNATAL LAMB

Jonathan Bensley, Victoria Stacy, Robert De Matteo, Richard Harding, M. Jane Black
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Background: Premature birth is the leading cause of perinatal morbidity and mortality. Little is known about how premature birth affects the development of the heart and in particular the growth of cardiomyocytes. This is an important area of study because cardiomyocytes cease dividing shortly after birth when they become terminally differentiated. If preterm birth leads to a reduced complement of cardiomyocytes this may affect the adaptive capabilities of the postnatal heart especially when challenged with induction of hypertrophy or following exposure to secondary insults

Hypothesis/Aims: We hypothesised that premature birth leads to accelerated maturation of cardiomyocytes and thus premature cessation of cardiomyocyte proliferation, resulting in a reduction in the number of cardiomyocytes in the heart of the preterm neonate. Therefore, the aim of this study was to estimate the number of cardiomyocytes in the left ventricle (with septum) and the right ventricle in the moderately preterm lamb heart at nine weeks of postnatal age.

Methods: Preterm delivery was induced in ewes at 133 days gestation (term =147 days) using Epostane (3-beta-HSD inhibitor), with the ewes of preterm lambs (n=7 also receiving a single sub-clinical dose of antenatal betamethasone to prevent respiratory distress. Term lambs (n=8) were used for comparison. All lambs were euthanised at 9 weeks post term equivalent age (PTEA) (a time point when cardiomyocytes have ceased proliferation). Cardiomyocyte number was estimated using an optical disector/fractionator method. Apoptosis detection was performed using the TUNEL reaction (ProMega DeadEnd Colourimetric Kit).

Results: At 9 weeks PTEA there was no significant difference in necropsy weight or heart weight in the preterm lambs compared to term lambs. There was also no significant difference in the total number of cardiomyocytes in the LV+S of the preterm lamb (n=5; $3.74 \times 10^9 \pm 3.12 \times 10^8$ cardiomyocytes) compared to term lambs (n=7; $3.83 \times 10^9 \pm 4.01 \times 10^8$ cardiomyocytes) at 9 weeks PTEA. Additionally, there was no significant difference in the total number of cardiomyocytes in the RV of the preterm lamb at 9 weeks of age PTEA (n=4; $1.19 \times 10^9 \pm 5.76 \times 10^8$) compared to term lambs at 9 weeks (n=5, $1.17 \times 10^9 \pm 6.97 \times 10^8$). There was a significant correlation between heart weight and the number of cardiomyocytes in the LV+S in both the term (r^2=0.553, p=0.05) and preterm (r^2=0.899, p=0.014) lambs. There were no differences between premature and term lambs in any of the morphometric parameters measured. There was no evidence of cardiomyocyte apoptosis in sections from the left ventricles in either the term or preterm animals.

Conclusion: Moderate premature birth does not appear to affect the number of cardiomyocytes in the preterm neonatal heart when postnatal growth is not compromised. However, since cardiomyocyte number is directly related to heart size it is likely that in preterm infants, where postnatal growth is compromised, that the complement of cardiomyocytes will be reduced.

SEXUAL DIMORPHISM OF CARDIAC MYOCYTES IN THE FETAL SHEEP AND THE EFFECTS OF IGF-1 TREATMENT

Min-Young Kim[1], Judith Burrell[1], Vasumathy Kumarasamy[1], Amanda Boyce[1], Karen Gibson[1], Kathy Gatford[2], Julie Owens[2], Eugenie Lumbers[1].
[1]Department of Physiology and Pharmacology, School of Medical Sciences, University of New South Wales, Sydney, Australia; [2] School of Obstetrics and Gynaecology, University of Adelaide, Adelaide, Australia.

Background: Insulin-like growth factors (IGFs) regulate proliferation and differentiation of many cell types throughout development.

Aims: To determine effects of IGF-I on the fetal heart.

Methods: IGF-1 was infused for 4 days (80 µg/h) into 8 chronically catheterised fetal sheep (n=8) aged 128 days. Control fetuses (n=11) were non-operated or vehicle-infused. Bromodeoxyuridine (BrDU, 250 mg in 16 mL i.v.) was infused on day 4; fetuses were killed at 135 days gestation. Cardiac myocytes were isolated from the left (LV) and right ventricular (RV) free walls, their individual volumes were measured and they were classified as uninucleate (UniNM) or binucleate (BiNM) using confocal microscopy, and according to the stage of the cell cycle by FACS.

Results: IGF-1 increased fetal kidney and spleen weights ($P<0.05$), while the increase in spleen weight occurred only in male fetuses (treatment * sex interaction, $P= 0.03$). IGF-1 did not increase total heart weight, but increased right ventricle weight/body weight (0.18 ± 0.01 compared with 0.16 ± 0.01 in controls, $P=0.04$). The systolic blood pressure of 7 IGF-1 treated fetuses was lower at the end of treatment compared to 6 control fetuses ($P<0.05$).
All 4 myocyte subtypes (uni- and binucleated from RV and LV) were larger in control female compared to male fetuses ($P<0.001$); while myocyte volumes were similar in control twins and singleton control fetuses. ANOVA, revealed interactions between IGF-1 treatment, sex and twinning, so post-hoc t-tests were carried out to determine if myocyte volumes were affected by IGF-1treatment in singleton and twin male and female fetuses.
Only in singleton male fetuses were the volumes of all 4 myocyte subtypes increased ($P < 0.007$). In female (singleton and twin) fetuses only the volumes of uninucleated RV free wall myocytes were increased ($P<0.01$).
IGF-1 did not alter the % of cardiac myocytes that were uninucleated. There was a lower percentage of uninucleated cells in the LV free wall of females compared to males ($P<0.05$).
IGF-1 reduced the % of RV cell nuclei in G0/G1, increased that in G2/M ($P\leq0.05$) and increased BrDU incorporation into RV nuclei ($P<0.05$). IGF-I also reduced the % of nuclei from the LV in S phase. ($P<0.05$).

Conclusions: Female fetal sheep have larger myocytes and a higher proportion of binucleated myocytes than male fetal sheep. This apparent greater maturity of myocytes in the female heart may be associated with increased contractility. IGF-1 has trophic effects on cardiac myocytes in the late gestation male singleton fetus only. Is this a reflection of their relative immaturity?

VITAMIN D INSUFFICIENCY AFFECTS CARDIOMYOCYTE NUMBER IN THE LEFT VENTRICLE OF 4 WEEK OLD SPRAGUE-DAWLEY RATS

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Departments of Anatomy & Developmental Biology[1], and Physiology[2], Monash University, Clayton, VIC.

Background: In recent years there has been a resurgence of vitamin D insufficiency in the community, particularly in women of child-bearing age. Vitamin D insufficiency during pregnancy may affect the development of the heart, since it is known to play an important role in cell proliferation and differentiation. Proliferation of cardiomyocytes occurs mainly prior to birth with postnatal growth of the heart predominantly due to cardiomyocyte hypertrophy. There is some evidence to suggest that vitamin D insufficiency leads to cardiomegaly.

Aim: The aim of this study was to determine in rat offspring the effect of exposure to vitamin D insufficiency from conception until 4 weeks of age on the development of the left ventricle.

Methods: Four week old Sprague-Dawley female rats were fed either a vitamin D deplete or vitamin D replete (control) diet for 6 weeks prior to pregnancy, during pregnancy and throughout lactation. Cardiomyocyte number was determined in the fixed left ventricle of offspring (*n* = 8/group) at 4 weeks of age, using an optical disector/fractionator stereological technique. In other litters, cardiomyocytes were enzymatically isolated from freshly excised left ventricles to determine the proportion of mononucleated and binucleated cardiomyocytes
(*n* = 4 litters/group).

Results: Cardiomyocyte number in the left ventricle of the vitamin D insufficient offspring was significantly increased (p= 0.01) compared with the control group in males (5.568 ± 0.256 x 10^7 cardiomyocytes and 6.308 ± 0.496 x 10^7 cardiomyocytes, respectively) and females (5.075 ± 0.491 x 10^7 cardiomyocytes and 6.465 ± 0.646 x 10^7 cardiomyocytes, respectively). This was accompanied by a significant delay in the maturation of the cardiomyocytes in the hearts of the vitamin D insufficient offspring (p=0.04).There was also a significant increase (p=0.005) in cardiomyocyte cross sectional area in the vitamin D insufficient group compared with control offspring.

Conclusion: Our findings suggest that exposure to vitamin D insufficiency in *utero* and early life leads to delayed maturation and subsequent enhanced growth (proliferation and hypertrophy) of cardiomyocytes in the left ventricle. This may lead to altered cardiac function later in life.

THE INTRAUTERINE GROWTH RESTRICTION-INDUCED DELAY IN CARDIOMYOCYTE BINUCLEATION IS RELATED TO HYPOXIA NOT HYPOGLYCEMIA

JL Morrison[1], KJ Botting[1,2], L Rattanatray[2], SJ Williams[2] and IC McMillen[1]
[1]Early Origins of Adult Health Research Group, Sansom Institute, University of South Australia and [2]Discipline of Physiology, University of Adelaide, Adelaide, South Australia, Australia

Background: Recent studies show that intrauterine growth restriction (IUGR) causes a delay in terminal differentiation of cardiomyocytes in the sheep fetus. The methods of inducing intrauterine growth restriction cause chronic hypoxia and hypoglycaemia but the underlying cause of the altered cardiomyocyte development remains unclear.

Aim: To investigate the role of hypoxia versus hypoglycaemia in cardiomyocyte development in the IUGR fetus.

Methods: Placental and hence fetal growth restriction was induced in fetal sheep by removing the majority of caruncles in the ewe before mating (placental restriction, PR). Vascular surgery was performed on 17 Control and 11 PR fetuses at 110-125d gestation (term=150±3d). PR fetuses with a mean gestational PO_2<17mmHg were defined as hypoxic. At *post mortem* (<135 or >135d), fetal hearts were collected, and cardiomyocytes isolated and fixed. Cardiomyocytes were stained with methylene blue to visualise the nuclei and the proportion of mononucleated cells was counted.

Results: PR resulted in chronic fetal hypoxia, IUGR, elevated plasma cortisol concentrations and reduced glucose concentrations. Although there was no difference in relative heart weights between Control and PR fetuses, there was an increase in the proportion of mononucleated cardiomyocytes in PR fetuses. There was a significant relationship between mean gestational PO_2 and the percentage of mononucleated cardiomyocytes in both the right and left ventricle. There was no relationship between plasma glucose and the percentage of mononucleated cardiomyocytes. Binucleated cardiomyocytes were relatively larger in PR compared to Control fetuses and this was inversely related to plasma glucose concentration.

Conclusions: The increase in the relative proportion of mononucleated cardiomyocytes in the growth restricted fetus is likely due to chronic hypoxia rather than hypoglycaemia.

AN INVESTIGATION INTO FETAL ARTERIAL STIFFNESS FOLLOWING MATERNAL ALCOHOL INFUSION IN SHEEP

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Background: Heavy alcohol consumption during pregnancy can lead to a well-known suite of characteristics, with fetal and neonatal growth restriction, physical abnormalities and intellectual impairment, known as fetal alcohol syndrome. However, there is little information on the effects of modest fetal alcohol exposure on the developing cardiovascular system.

Aim: To investigate the effects of fetal alcohol exposure in the last third of gestation in sheep on arterial stiffness and collagen and elastin content in vessels from selected major organs.

Methods: Under general anaesthesia, pregnant ewes at 91±1 days after mating (D91±1) were implanted with carotid artery and jugular vein catheters for infusing alcohol and assessing plasma alcohol levels. Daily alcohol infusions (0.75g/kg over 1 hr) began at D95±1 and continued to D133±1. Five sheep were ethanol infused and 5 saline infused sheep served as controls. Ewes and fetuses were killed at D134 and the fetal heart, brain, kidney, small intestine and psoas muscle were removed into ice-cold physiological saline. Small resistance arteries (250-350μm outside diameter) were dissected from these tissues. We determined (1) arterial stiffness using a pressure myograph and (2) changes in mRNA expression in *collagen Iα1, collagen Iα2, collagen III, tropoelastin* and *elastase 2* using real time PCR.

Results: Plasma alcohol concentrations peaked at 0.12±0.01 g/dL in both the ewe and the fetus at the end of the 1 hr infusion. Levels declined to basal within the following 8 hrs. Pressure-diameter relationships were reduced in the coronary, cerebral, renal, mesenteric and psoas arteries of alcohol exposed fetuses ($p=0.0001$), indicating increased vascular stiffness. Real time PCR showed increased expression in *collagen Iα1* in cerebral ($p=0.05$), renal ($p=0.05$) and mesenteric ($p=0.04$) vessels, and in *tropoelastin* in cerebral vessels ($p=0.0001$). There were no changes in mRNA levels in the coronary vessels.

Conclusions: Alcohol exposure during late gestation results in widespread increases in arterial wall stiffness, involving a variety of arteries in the fetus. This was associated with increases in *collagen* and *tropoelastin* mRNA within these vessels. These effects may have long lasting consequences for vascular function.

SEX DIFFERENCES IN THE PROGRESSION OF CARDIOVASCULAR DISEASE IN SHEEP FOLLOWING FETAL UNILATERAL NEPHRECTOMY

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Background: It is proposed that a reduction in nephron number may predispose to renal impairment and development of hypertension. Furthermore, the incidence of cardiovascular mortality is reported to be higher in populations with renal disease and sex differences appear to play a role in the onset and progression of these diseases.

Objective: This study investigated the effects of fetal uninephrectomy (uni-x) in sheep during the active period of nephrogenesis on kidney and cardiovascular function in male and female sheep at 6 months and then at 24 months of age.

Methods: Fetal uni-x or sham surgery was performed at 100d of gestation (term=150 days). At 6 and 24 months of age basal mean arterial pressure (MAP) was measured via a carotid artery catheter over 72 hours in conscious animals. Renal function was determined by measurement of urinary protein excretion rates in all animals. Cardiac structure was assessed through echocardiography measurements performed in conscious sheep at 24 months of age.

Results: Data are summarized in the table. MAP was significantly elevated in both uni-x male and female sheep at 24 months of age as compared to the sham animals but the onset of hypertension was observed earlier in uni-x male sheep than the female sheep ($P_{treatment}<0.001$, $P_{sex}<0.001$, $P_{age}<0.01$). Urinary protein excretion rate was significantly elevated in uni-x animals at 24 months of age, however, only the uni-x males had evidence of proteinuria at 6 months of age. Interestingly, the sham males also had elevated proteinuria at 24 months of age. Uni-x males had significantly greater left ventricular mass (LV mass, $P<0.001$)), reduced fractional shortening (FS, $P<0.001$) and reduced ejection fraction (EF, $P<0.001$) at 24 months of age compared to the sham group and females. No incidence of ventricular hypertrophy was observed in uni-x females at 24 months of age.

		Sham		Uni-x	
		Male	Female	Male	Female
MAP (mm Hg)	6 month	76±2	79±2	91±4	82±2
	24 month	78±3	80±5	105±2	103±10
Urinary protein excretion (g/d)	6 month	0.32±0.07	0.32±0.01	0.42±0.06	0.31±0.02
	24 month	0.47±0.001	0.33±0.02	0.71±0.02	0.38±0.01
LV mass (g)	24 month	98±10	84±9	196±15	79±2
% FS	24 month	64±6	61±5	47±3	60±5
% EF	24 month	34±5	32±4	23±2	31±3

Conclusion: Fetal uni-x results in renal impairment and elevated arterial pressure in male and female sheep. However, the onset of disease appears to be earlier in males then females. The effects of ageing, at least with regards to renal function appear, to be more pronounced in males than females. The onset of cardiovascular disease also appears to be earlier in males as observed by the presence of left ventricular hypertrophy in uni-x males.

NOTES

ASSESSMENT OF AUTONOMIC CARDIOVASCULAR MARKERS AS INDICATORS OF CLINICAL COMPROMISE IN ILL PRETERM INFANTS DURING THE FIRST DAYS OF LIFE

Vera Golder, Rosemary SC Horne
Ritchie Centre for Baby Health Research, Monash Institute of Medical Research, Monash University, Melbourne, Australia.

Background: The first days after preterm birth are a critical period of instability for infants as the circulation changes from fetal to neonatal configuration. In addition, autonomic control of the cardiovascular system is immature, increasing the risk for unstable heart rate (HR) and mean arterial blood pressure (MAP), and cardiovascular compromise. We aimed to examine autonomic cardiovascular control in preterm infants in the first days of life by measuring HR and MAP, and calculating heart rate variability (HRV) and baroreflex sensitivity (BRS) as markers of autonomic function; and to assess whether these variables could be used as indicators of clinical compromise.

Methods: Infants (N=14) born between 23-35 weeks gestational age (GA) with indwelling arterial catheters and no congenital abnormalities were recruited from the NICU and studied longitudinally (N=43 studies) for 1-8 days following birth. MAP and HR were recorded beat-beat and HRV and BRS analysed in the time domain. A clinical risk index for babies (CRIB score), a 12 h measurement of mortality risk that is independent of blood pressure, was calculated and compared to cardiovascular variables. Cardiovascular and autonomic variables were compared to the CRIB score calculated 12 h prior, during and after each study.

Results: MAP was negatively correlated with the CRIB scores calculated 12 h prior to and post each study ($p<0.05$; $p<0.05$ respectively), but not with the CRIB scores calculated 12 h during the study. HR did not have a significant correlation with any CRIB score. Short term HRV, indicative of parasympathetic modulation, had a significant negative correlation with the CRIB scores calculated 12 h prior to each study ($p<0.01$), but not with the CRIB scores calculated during or after each study. Long term HRV, indicative of predominantly sympathetic modulation, had a significant negative correlation with the CRIB scores calculated 12 h prior to and post each study ($p<0.01$; $p<0.05$ respectively), but not with the CRIB scores calculated 12 h during the study. BRS had a significant negative correlation with the CRIB scores calculated 12 h prior, during and post each study ($p<0.05$; $p<0.05$; $p<0.05$ respectively).

Conclusions: The strongest correlation between cardiovascular variables as markers of autonomic function was with the CRIB scores calculated 12 h prior to each study. This suggests that autonomic cardiovascular control declines after the risk of mortality increases, and therefore suggests that preterm infants may be unable to maintain their MAP and HR and stable levels in the face of a worsening clinical status.

DEEP-HYPOTHERMIC CIRCULATORY ARREST DURING THE ARTERIAL SWITCH OPERATION IS ASSOCIATED WITH ABNORMAL INTRACEREBRAL OXYGENATION IN THE EARLY POST-OPERATIVE PERIOD

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[1]Department of Physiology, Faculty of Medical and Health Sciences, University of Auckland, [2]Paediatric Intensive Care Unit, Starship Children's Hospital, Auckland, New Zealand

Background: Transposition of the Great Arteries is the most common form of cyanotic congenital heart disease, affecting around 2/10000 live births. Deep-hypothermic circulatory arrest during corrective surgery is associated with delayed seizures and a higher risk of developmental abnormalities.

Hypothesis: That circulatory arrest is associated with abnormal cerebral oxidative metabolism and EEG abnormalities in the early post-operative period.

Methods: Near-infrared spectroscopy (NIRS) parameters, arterial oxygen saturation (SaO2), and mean arterial blood pressure (MAP) were recorded from 4 to 15 h after surgery. Intracerebral oxygenation was determined as the tissue oxygenation index (TOI) and fractional tissue oxygen extraction (FTOE = [SaO2 – TOI] / SaO2).

Results: Both groups showed a significant overall rise in TOI and fall in FTOE over the early post-operative period. Patients exposed to deep-hypothermic circulatory arrest had a significantly lower mean arterial pressure (mean ± SD, 47.9 ± 3.6 *vs.* 52.3 ± 2.2 mmHg, 4-15h post-bypass, $p<0.05$), lower fractional tissue oxygen extraction (0.41 ± 0.02 *vs.* 0.47 ± 0.03, $p < 0.05$) and transiently higher tissue oxygenation index (57.9 ± 2.0 *vs.* 52.7 ± 2.5%, $p<0.05$, 4-8 hours after bypass) compared to patients who had low-flow cardiopulmonary bypass alone.

Conclusions: These data suggest that even the relative brief periods of circulatory arrest now used during the arterial switch operation are associated with impaired cerebral oxidative metabolism leading to reduced oxygen uptake and greater intracerebral oxygenation during the acute recovery period.

EFFECT OF PREMATURE BIRTH ON HEART RATE AND BLOOD PRESSURE DURING SLEEP

Nicole B Smith, Stephanie R Yiallourou, Adrian M Walker, Rosemary SC Horne
Ritchie Centre for Baby Health Research, Monash Institute of Medical Research, Monash University, Melbourne, Australia.

Background: Infants born prematurely are at an increased risk for Sudden Infant Death Syndrome (SIDS). It has been suggested that this may be due to immature autonomic control of heart rate (HR) and blood pressure (BP). Previous studies have identified that preterm infants have immature HR and BP control at term-equivalent age, however little information is available beyond this age. To date, there is a paucity of data on BP and its control during sleep in preterm infants. Investigation of both HR and BP across the age of greatest SIDS risk may aid in understanding the pathogenesis of SIDS.

Aims: To determine the effect of premature birth on HR and BP control during sleep over the first 6 months of life after reaching term-equivalent age.

Methods: Twenty-seven preterm infants (28-32 wk GA) and 20 term infants (38-42 wk GA) were studied using daytime polysomnography at 2-3 wk, 2-3 mo and 5-6 mo corrected age (CA). BP was recorded using a photoplethysmographic cuff (Finometer™). BP and HR were assessed during both quiet (QS) and active (AS) sleep.

Results: HR was lower during QS compared to AS at 5-6 mo CA and fell with increasing postnatal age ($p<0.05$). BP was lower in QS compared to AS at all ages studied ($p<0.001$) and BP tended to be lower at 2-3 mo CA compared to both 2-4 wk and 5-6 mo CA. Comparison of preterm and term groups identified that BP was lower in the preterm group during QS and AS at all ages studied ($p<0.05$).

Conclusion: This was the first study to provide normative data on the maturation of HR and BP during sleep in prematurely-born infants. We have identified marked differences in BP between preterm and term infants, suggesting that cardiovascular control is altered in preterm infants and may contribute to the increased incidence of SIDS in this group.

COMPARISON OF THE RELATIONSHIP BETWEEN CEREBRAL PERFUSION AND CARDIAC FUNCTION IN ELBW INFANTS AND VLBW INFANTS

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Background: To stabilize the systemic circulation in extremely low birth weight (ELBW) infants in the immediate postnatal period is important to manage condition affecting for their central nervous system morbidity and neurodevelopmental outcome. Near-infrared spectroscopy (NIRS) provides information on both cerebral oxygenation and cerebral blood flow (CBF). Superior vena cava (SVC) flow assessment by echocardiography has been reported to be a predictive marker of intraventricular hemorrhage (IVH) in preterm infants.

Aims: The present study evaluated the relationship between cerebral perfusion and cardiac function by using both NIRS and echocardiography, comparing ELBW infants with very low birth weight (VLBW) infants.

Methods: The tissue oxygenation index (TOI) using NIRS was monitored in the front temporal region at 3-6, 12, 18, 24, 36, 48 and 72 hours after birth in VLBW infants. Serial echocardiographic assessments were performed simultaneously. The end-systolic wall stress (ESWS), mean velocity of circumferential fiber shortening (mVcfc), left ventricular fractional shorthening (LVFS), LV output (LVO), and SVC flow were measured using M-mode and Doppler echocardiography. Heart rate, mean blood pressure, SaO_2, and PCO_2 were also recorded. Statistical analyses were performed for all findings.

Results: The subjects were 12 ELBW infants and 12 VLBW infants had a mean gestation of 25.7 ± 1.5 weeks and 29.0 ± 1.4 weeks, and a mean birth weight of 793.6 ± 166.0 g and 1268.6 ± 145.2 g, respectively. There were no significant differences in heart rate, SaO_2, and PCO_2. Mean blood pressure in ELBW infants significantly increased gradually after birth. The ESWS increased markedly at 18 hours after birth and decreased gradually in both groups. The mVcfc, LVFS, LVO, SVC flow and TOI decreased transiently within 24 hours, and then increased gradually, especially in ELBW infants. All minimum values of these indices in ELBW infants was lower than in VLBW infants. The LVO and SVC flow showed significant differences in the two groups.

Conclusions: Changes in cardiac functions reflected changes in SVC flow and TOI, reflecting cerebral perfusion. Changes in blood pressure in ELBW infants soon after birth did not reflect the changes in these indices. Assessing early circulatory status using echocardiography appears useful for the management of cerebral circulation, especially in ELBW infants.

SWADDLING INFANTS: EFFECTS ON AROUSABILITY FROM SLEEP

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Background: It has been proposed that an impaired ability to arouse from sleep may be involved in the pathogenesis of the Sudden Infant Death Syndrome (SIDS). Currently, swaddling is promoted by some Australian SIDS organisations as a method for settling infants in the supine position, aiming to reduce the incidence of infants being placed prone for improved sleep.

Aim: To evaluate the effects of swaddling infants on spontaneous arousability from sleep as a test of its safety.

Methods: 26 healthy term infants who were routinely swaddled (n=15) and unswaddled (n=12) at home were studied. Infants were studied with daytime polysomnography, in both swaddled and non-swaddled conditions, at both 3-4 wk and 3 mo after birth. Arousal from sleep was induced using a pulsatile jet of air to the nostrils at increasing pressures (results not reported). Spontaneous arousals were also observed during uninterrupted sleep between tests; these were scored as sub-cortical activations (SCA) and cortical arousals (CA) using standard criteria and expressed per hour spent in each condition. Two-way RM ANOVA was used to assess effects of sleep state/swaddling and postnatal age on the frequency of total spontaneous arousals, SCAs and CAs.

Results: Regardless of sleep state and age, swaddling had no significant effects on baseline physiological variables (heart rate, oxygen saturation and abdominal skin temperature). In all sleeping conditions at all ages studied, significantly more arousals were observed during active sleep (AS) compared with quiet sleep (QS). Overall, infants tended to arouse less frequently when swaddled, however this failed to reach statistical significance. During AS at 2-3 wk of age,, infants exhibited fewer CAs when swaddled (3.8 ± 0.8/h) compared with unswaddled (5.7 ± 0.8/h, $p<0.05$). There were no significant effects of swaddling on the frequency of SCAs at either age studied. In AS, when unswaddled, there were increased total spontaneous arousals (14.2 ± 1.5/h vs 10.7 ± 1.1/h, $p<0.05$) and SCAs (8.2 ± 1.3/h vs 4.9 ± 0.7, $p<0.05$) at 3mo compared with 3-4 wk
There were no significant differences in total arousability between routinely swaddled and unswaddled infants in either sleep state at either age. However, at 3 mo in the routinely unswaddled group only. swaddling during AS was associated with a decrease in spontaneous CAs (4.7 ± 1.3/h, $p<0.05$) when compared with non-swaddled (6.1 ± 1.1/h),

Conclusions: This study has demonstrated that overall, swaddling did not affect infant arousability or any of the physiological variables recorded. In addition, the finding that there was no effect of routine swaddling on overall arousability supports the contention that infant swaddling is a safe means to settle infants and improve sleep in the supine position.

GLUCOCORTICOID REGULATION OF PROSTAGLANDIN SYNTHESIS IN THE HUMAN FETAL MEMBRANES: DUALITY IN THE STYLE OF DR. JEKYLL AND MR. HYDE?

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Background: Glucocorticoids can either stimulate or repress the expression of glucocorticoid responsive genes. Several years ago we have discovered that glucocorticoids stimulate Prostaglandin Synthase-2 (PGHS-2, COX-2 or PTGS2) expression in *primary amnion cell cultures* (Zakar & Olson J. Dev. Physiol. 1989 12:269-72) (Zakar, Hirst et al., Endocrinology 1995 136:1610-9). Others have confirmed this observation and extended it to cultured chorion cells. PGHS-2 is the key enzyme of prostaglandin biosynthesis in the fetal membranes; therefore it was proposed that cortisol promotes parturition in women by stimulating the intrauterine production of labour-inducing prostaglandins as in the sheep. Glucocorticoid administration to pregnant women, however, does not increase myometrial contractility and does not affect gestational length. The mechanism of the stimulation is also unclear, because the human PGHS-2 gene promoter does not contain a palindromic glucocorticoid response element (GRE), which mediates the glucocorticoid up-regulation of responsive genes. Moreover, we have also observed that glucocorticoids inhibit PGHS synthesis and activity in *explants of freshly isolated amnion* (Zakar, Teixeira, Hirst et al., J. Reprod. Fertil. 1994 100:43-50). It is therefore unclear which of these opposing actions dominates *in vivo* and how is the duality of glucocorticoid action regulated in normal pregnancy and in preterm labour.

Aims/Hypothesis: We wished to determine whether glucocorticoids stimulate or inhibit PGHS-2 gene expression in the fetal membranes *in vivo*.

Methods: Amnion tissue explants from term pregnancies (delivered in the absence of labour) were incubated in serum-free medium for 24h with or without dexamethasone. PGHS-2 gene activity and mRNA levels were determined by real-time RT-PCR. Glucocorticoid receptor binding to the PGHS-2 promoter was measured by chromatin immunoprecipitation.

Results: PGHS-2 mRNA levels increased spontaneously after 24h of incubation. Dexamethasone (4 - 100nM) completely and dose dependently abolished the spontaneous increase of mRNA levels and decreased PGHS-2 gene activity. Chromatin immunoprecipitation showed glucocorticoid receptor binding to the 1 kb upstream promoter region of the PGHS-2 gene, where NF-kappaB and AP1 transcription factor binding sites are present.

Conclusions: The PGHS-2 gene is chronically suppressed *in vivo* by endogenous glucocorticoids. The mechanism possibly involves the transrepression of promoter-bound NF-kappaB and/or AP1 transcription factors by the glucocorticoid receptor. Chronic glucocorticoid suppression may protect the pregnancy from the early up-regulation of prostaglandin production in the uterus (Dr. Jekyll). We speculate that pathological dysregulation may result in the dominance of stimulatory glucocorticoid action on PGHS-2 gene activity and enzyme levels, increasing the risk of preterm labour (Mr. Hyde). Synthetic glucocorticoids with selective transrepressor activity (the “SEGRA” compounds) may inhibit PGHS-2 expression in the fetal membranes and might be useful for the prevention of preterm birth

NOTES

MODULATION OF T CELL SUBSETS ASSOCIATED WITH INTRA-AMNIOTIC UREAPLASMA IS DAMPED BY MATERNAL PROGESTERONE AND BETAMETHASONE TREATMENTS

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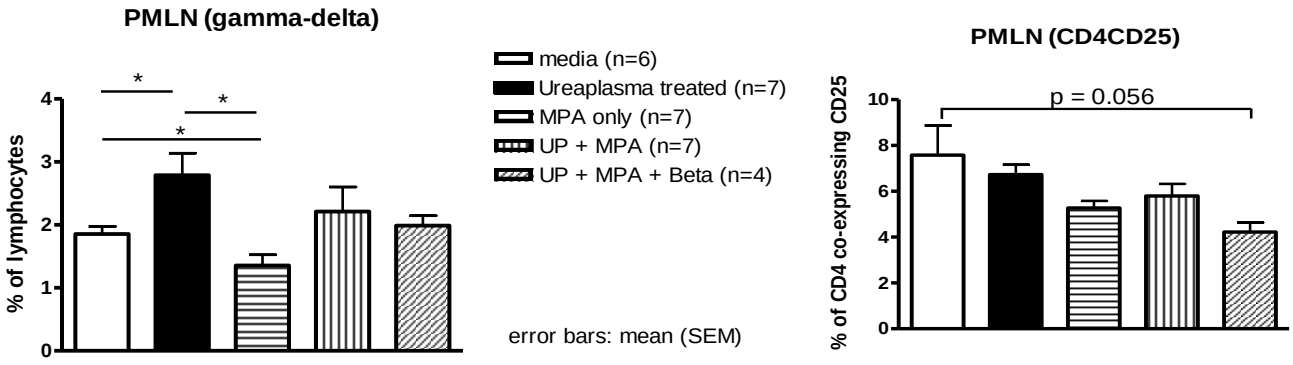
Background: Ureaplasma infections are associated with chorioamnionitis, preterm delivery and preterm lung disease. Women in preterm labour are given betamethasone to induce fetal lung maturation. We hypothesised that antenatal exposure to ureaplasma alters fetal innate immunity, and that this response may be modulated by maternal betamethasone and progesterone treatments.

Methods: Date-mated ewes received $2x10^7$ cfu *Ureaplasma parvum* (UP) or an equivalent volume of media (controls) intra-amniotically at 80 d gestation. Maternal medroxyprogesterone acetate (MPA) and/or betamethasone were administered intra-muscularly at 110 d and 117 d respectively. Lambs were delivered at 124 d (term = 150 d); the thymus and posterior mediastinal lymph node (PMLN) were removed at postmortem and mechanically dissociated into single-cell suspensions. The expressions of CD8, CD4, CD25 and T-cell receptor 1 (gamma-delta T cells: γδ) cell-surface markers were assessed via flow cytometry. Statistical analysis was calculated using student's t-test.

Results: Compared to controls, γδ T cell expression in the PMLN was increased in the UP group (p=0.04) but decreased in the MPA group (p=0.043). There was a trend to decreased % of CD4 T cells co-expressing the activation marker CD25 in lambs exposed to IA ureaplasma and maternal MPA and Beta.



Conclusions: IA ureaplasma modulates T cell subsets in the lymph node draining the lung (PMLN). Maternal administration of MPA/Betamethasone may dampen the T cell subset response to Ureaplasma.

Support: NIAI, Viertel SMRF (JJP), NHFA/NHMRC Fellowship (GRP)

NEUROSTEROID PROFILES IN FETAL, MATERNAL AND NEONATAL GUINEA PIGS USING GAS CHROMATOGRAPHY-MASS SPECTROMETRY.

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Background: Placental progesterone production has a major role in regulating fetal CNS activity. This action is mediated by neuroactive metabolite steroids such as allopregnanlone. These steroids are potent GABAA receptor agonists and have been shown to have a key role in reducing neuronal excitotoxicity through the GABAergic inhibition of CNS excitability. The relatively high levels of neurosteroids in the fetus and mother may be involved in protecting the fetus from brain injury during compromised pregnancies. However, the overall action of these steroids in determining CNS excitability depends on the relative levels of pregnanolone isomers and the profiles of these isomers may change markedly in the neonate after loss of the placenta.

Aims: The aims of this study were firstly to optimise and validate a method for the simultaneous identification and measurement of four pregnanolone isomers in the guinea pig using Gas Chromatography-Mass Spectrometry. Secondly to evaluate neurosteroid isomer levels in the maternal circulation and fetus and changes in isomer profile after birth.

Methods: Steroids were isolated from plasma samples in 85% methanol fractions following C18 solid phase extraction. Dried steroid extracts were derivatised to form tri-methylsilyl (TMS) derivatives prior to GC/MS analysis in an optimised reaction using a combination of Methyloxime (MOX) and the silylation reagent BSTFA + TMCS (99:1). Steroid analysis was performed using a Quadrupole GC/MS system with electron-impact ionisation. GC separation was performed using a Phenomenex® Zebron ZB-50, 30m x 0.25mm column with a 0.25um film thickness. MS analysis was performed using optimised program retention time and ion mass detection settings for each steroid of interest. Concentration measurements were performed using two effective ion masses (m/z), the first used in quantification and the second used to improve compound identification. All samples were analysed with a known concentration of internal standard (ISTD).

Results: Optimal separation of pregnanolone isomers was achieved using a Zebron ZB-50 column combined with Selective Ion Monitoring (SIM) mode analysis. Steroids were fully resolved with the following optimal retention times recorded: Epipregnanolone - 28.96 min, Allopregnanolone - 29.29 min, Pregnanolone - 29.86 min, Isopregnanolone - 13.84 min and Alfaxalone - 33.98 min (ISTD). In the maternal circulation, allopregnanolone concentration ranged from 5.09 - 5.76 nmol/L, pregnanolone 7.11 - 8.94 nmol/L and isopregnanolone 23 µmol/L while epipregnanolone was at the limit of detection. In contrast, in the neonate isopregnanolone levels remained high, whereas 3alpha isomer levels were lower (pregnanolone, 4.88 nmol/L) and the other isomer levels were at the limit of detection.

Conclusions: Both 3alpha and 3beta isomers were present in the maternal circulation suggesting the balance of GABAA receptor active and inactive isomers affect the fetus. Inactive 3beta isomers predominated in the neonatal plasma. The higher levels of active isomers before birth, is consistent with a role of these progesterone metabolites in influencing fetal CNS activity.

DOES CHORIOAMNIONITIS INFECTION AFFECT NEPHRON FORMATION IN THE HUMAN FETAL KIDNEY?

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Background: Preterm birth accounts for approximately 8% of all live births in Australia. Chorioamnionitis is an intrauterine bacterial infection that affects the fetal membranes and/or amniotic fluid, and is known to be one of the major causes of preterm birth. Chorioamnionitis has been associated with fetal injury and is linked to the development of chronic lung disease and brain damage. However, the effects of chorioamnionitis on the kidney, in particular nephrogenesis (the formation of nephrons) and renal morphology are as yet unknown.

Aims: This study aims to investigate the effect of chorioamnionitis on the developing human kidney at different time points in gestation. The specific aim was to determine the effect of exposure to chorioamnionitis on renal morphology and the number of glomerular generations in the developing fetal kidney.

Methods: Archived paraffin blocks of kidney tissue and autopsy reports from stillborn infants were obtained from the Women's and Children's Hospital in North Adelaide, South Australia. In all cases consent for research had been given. A total of 42 kidneys from singleton fetuses from varying gestational ages (20wks – 40wks) with the cause of death given as chorioamnionitis were examined. Kidneys from age-matched stillborn fetuses (n=37) with an unexplained cause of death were used for comparison. Medullary ray glomerular counts were undertaken on 5µm paraffin sections stained with haematoxylin and eosin; at least 4 different medullary rays per kidney were analysed and glomerular generation counts for each section were averaged. The investigator was blinded to the experimental category whilst performing the analysis.

Results: There were no apparent abnormalities in the renal morphology of fetuses exposed to chorioamnionitis compared with age-matched controls. At different gestational time-points there were no differences in the number of glomerular generations formed in the kidneys of the fetuses exposed to chorioamnionitis compared with gestational age-matched controls. Significant correlations were found between birth weight and kidney weight with the number of glomerular generations – in both the chorioamnionitis group (r^2=0.636, P< 0.0001, r^2=0.700, P<0.0001 respectively) and control group (r^2=0.786, P<0.0001, r^2=0.795, P<0.0001 respectively).

Conclusions: Exposure of the fetus to chorioamnionitis does not appear to affect nephrogenesis in the developing kidney. In the control kidneys and in the kidneys exposed to chorioamnionitis both kidney size and fetal birth weight were strong determinants of the number of glomerular generations.

EFFECTS OF INTRA-AMNIOTIC ENDOTOXIN ON POSTNATAL ORGAN GROWTH IN RATS

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Background: Intrauterine infection/inflammation (*ie* chorioamnionitis) is widely recognized as one of the most important factors causing preterm delivery. It is associated with impairment of various fetal organs such as brain (white matter injury) and lungs (chronic lung disease).

Aim: To study the effects of antenatal intra-amniotic lipopolysaccharide (LPS), a potent proinflammatory agent, on growth and development of various organs (brain, lungs, heart, kidneys) in newborn rats.

Methods: At 20 d gestation, pregnant SD rats were anesthetized and the uterus exposed under general anesthesia. The uterine wall was punctured and 0.1-0.2μg LPS; *E. coli* endotoxin (O55:B5; Sigma, St. Louis, MO, USA) dissolved in 0.1mL saline injected into each amniotic cavity. In the control group, 0.1mL saline was injected. At 22 d (term), the fetuses were delivered spontaneously and vaginally. The newborn pups were nursed by their own mother. After birth at 0, ~3, ~7, ~14, ~28 d, the pups were euthanized and the organs (brain, lungs, heart, kidneys) were weighed and fixed/frozen for further analysis.

Results: There were no significant differences in body weight over study period. The weights of the brain, heart, lungs relative to body weight were the highest at ~7 d. The kidney/body weight ratio was the highest at ~14 d. In LPS treated rats compared to controls, the organ to body weight ratios tended to be lower in the brain, kidneys, and lungs at ~7 to ~14 d.

Weights at 14 days	Control	LPS
Body weight (grams)	29+/-4	34+/-2
Brain/body weight ($x10^{-3}$)	29+/-2	23+/-1*
Rt lung/body weight ($x10^{-3}$)	9.6+/-0.8	8.6+/-0.6
Heart/body weight ($x10^{-3}$)	6.1+/-0.6	5.9+/-1.1
Rt Kidney/body weight ($x10^{-3}$)	6.4+/-0.5	6.2+/-0.3

*p <0.05

Conclusions: Antenatal intra-amniotic LPS 0.1-0.2μg did not affect fetal and neonatal body weight gain. However it may influence perinatal organ growth and development. Structural and biochemical analyses are underway.

EFFECTS OF ENDOTOXIN IN FETAL SHEEP: IS ERYTHROPOIETIN PROTECTIVE?

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Background: Intrauterine inflammation and infection have been linked to preterm birth and brain injury in the newborn. Exposure of fetal sheep to the bacterial endotoxin lipopolysaccharide (LPS) induces intrauterine inflammation and has been shown to cause brain injury (Duncan et al 2002) and damage to the placenta (Duncan et al 2003) We have tested the ability of agents such as erythropoietin (EPO) to alleviate injuries induced by intrauterine inflammation in the brain and other organs.

Aims/Hypothesis: To determine the effectiveness of EPO in ameliorating organ damage and physiological effects induced by fetal exposure to LPS in sheep.

Methods: At 0.7 of gestation (term ~147 days) chronically catheterized fetuses received either: i) bolus dose of LPS (~ 0.9µg/kg, i.v.) on 3 consecutive days (n=8); ii) i.v. bolus of LPS followed 1 hour later by 5000IU of recombinant human Epo (rhEpo; n=15); iii) rhEpo alone (n=5); or iv) saline alone (n=5). Fetal arterial blood gases and arterial pressure were monitored. Six days after the last LPS exposure, ewes and fetuses were euthanased, and fetal brain, fetal liver and placenta were examined histologically.

Results: Administration of saline alone or rhEpo alone did not alter fetal arterial blood gases, blood pressure or damage the fetal brain, liver or placenta. Repeated LPS exposure resulted in fetal hypoxemia, increased plasma haematocrit and brain damage including white matter injury ranging from cystic infarction to extensive, diffuse axonal damage and microgliosis. Placental and liver damage was evident in 50% of LPS treated fetuses. Fetuses treated with LPS plus rhEpo become significantly more hypoxemic, displayed higher plasma lactate levels, became more hypotensive and tended to be tachycardic than fetuses treated with LPS alone. However brain injury and damage to liver and placenta were reduced.

Conclusions: Treatment of fetal sheep repeatedly exposed to LPS with rhEpo worsened the physiological status of the fetus and increased the likelihood of fetal death. However, rhEpo treatment limited the extent of brain injury and also appeared to protect the placenta and liver. EPO may exert its actions through anti-inflammatory and/or anti-apoptotic pathways. Further studies are needed to elucidate the beneficial action of EPO.

Duncan et al (2002) *Pediatric Research* 52, 941-949.
Duncan et al (2003) *Placenta* 24; 786-789.

NOTES

DIFFERENTIATING THE INFLUENCE OF GESTATION LENGTH AND BIRTHWEIGHT PERCENTILE ON MOTOR CORTICAL DEVELOPMENT IN CHILDREN

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Background: The highest prevalence of motor and cognitive disabilities is in very preterm children, but the *greatest burden* of disability may lie in children born at 32-37 weeks, the largest group of all preterm children. The few follow-up studies of this gestation group show that children who were apparently neurologically normal during infancy and preschool years exhibit significant motor, cognitive and behavioural deficits at school age. The neurophysiology underlying the motor developmental delay or dysfunction is unknown.

Hypothesis: Hitherto undetected alterations in the normal development of the motor cortex (M1) and the corticospinal pathways underpin at least some of the motor dysfunction seen preterm children. We also aimed to differentiate the relative influences of gestation length (GA) from fetal growth (i.e. birthweight expressed as a percentile for gestational age; BW%) across the range of survivable gestations.

Methods: 72 children (age: 11.4 yrs ± 5.0 mths, 40 males) born 26-40 wks gestation were recruited from WCH databases. Transcranial magnetic stimulation (TMS) and electromyography was used to assess a range of intracortical and interhemispheric M1 functions and the stimulus-response (S-R) characteristics of the corticospinal projection to the hands.

Results: The prevalence of left handedness increased with shorter GA but was not influenced by BW% or gender. Lower GA was correlated with a higher resting motor threshold (rMT: i.e. the lowest TMS stimulus intensity required to activate the corticospinal pathway) for both hands (Right: r=-0.39, p=0.001; Left: r=-0.42, p≤0.0001) and a smaller area under the curve for the right hand only (r=0.64, p=0.008). For every week GA shortened, the stimulator intensity required to activate the corticospinal projection increased by 1.6% for the right hand and 1.4% for the left hand. Adult rMTs were only consistently found in children born ≥ 38 wks GA. Low BW% predicted a high rMT (r=-0.46, p=0.04) and a smaller area under the curve for the left hand only (r=0.61, p=0.04). For every 1% BW% decreased, the stimulator intensity required to activate the projection to the left hand increased 0.1% independently of GA. Transcallosal inhibition of the left M1 by the right M1 was negatively correlated with age (r=-0.45, p=0.01) and BW% (r=-0.38, p=0.02). Intracortical inhibition and facilitation was not altered by age, GA or BW%.

Conclusions: Children start exhibiting adult rMT values (~30-50% of stimulator output) by 10-11 yrs of age and most have adult rMTs by 13 yrs. rMT reflects the excitability properties of the cortical and spinal motoneuron pools. Our preliminary findings suggest that, in the 10-12 yr age group, adult rMTs tend only to be evident in children born with a GA ≥38 wks. It is unclear yet whether this reflects developmental delay or more permanent change. The larger the area under the SR curve, the stronger the neural projection e.g. highly dextrous hand muscles have much stronger projections than leg or trunk muscles. Development of the projection from the left M1 to the right hand appears to be either delayed or permanently altered by preterm birth and this may explain their greater prevalence of left-handedness. The effect of low BW% appears to be confined to the right M1. Transcallosal inhibition of the M1s, important for bimanual tasks, does not appear mature in this age-group, although it may also be reduced by low BW%, possibly due to delayed/faulty right M1 development. Intracortical excitability development may be more dependent upon post-natal factors such as activity and opportunity.

INQUIRY INTO THE MECHANISMS OF FOLDING OF THE CEREBRAL CORTEX DURING FETAL DEVELOPMENT IN SHEEP

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Background: Folding of *gyrencephalic* brains allows the cortical mantle to have a large surface area while minimizing overall brain volume. The major cortical folds appear gradually during development, in humans from about 16 weeks of pregnancy, and in sheep from about 60 days gestation (about 40% of full gestation). The first convolutions to appear, the primary inward folds of the central and calcarine sulci, are relatively invariant in position and eventual depth, but the subsequent secondary and tertiary folding is more variable. The major gyri (the outward surface of such folding) appear to be fully developed by term, but in some species, including humans, some further tertiary folding continues after birth.

Aims/Hypothesis: The mechanisms underlying the development of cortical folding is still poorly understood. We examined the possibility that the cortex folds because it expands tangentially, and that this expansion occurs between predetermined 'nodes' present either at the cortical surface, or at the boundary between white matter and gray matter.

Methods: Brains were obtained from fetal sheep between 60 and 130 days gestation (DGA), and perfusion-fixed with 4% paraformaldehyde. T2-weighted and 12-direction diffusion weighted MR images were acquired using a 4.7 T Bruker Biospec scanner (Geng et al, 2008). Mean cortical thickness, cortical surface area, and the number of sulcal folds were calculated in a restricted region. Fractional anisotropy (FA) was calculated from the DTI data throughout the white matter and cortical mantle. FA is a measure of the directional restriction of water molecule diffusion that, in the brain, largely results from the development of directionally-uniform fibre tracts.

Results: The surface of the brain at 60 and 70 DGA was smooth with only a few shallow dimples. The number of sulci increased from approx 6 to 45 between 70 and 130 DGA; the greatest increase occurred between 80 and 90 DGA when there was only a minimal change of cortical thickness (2.2 to 2.4 mm) but a 2-fold increase of cortical surface area (4000 to 8000 mm^2). Given the restricted space between the developing white and subcortical gray matter and the skull, the tangential growth of the cortex is hypothesised to lead to the formation of gyri and sulci. The development of white matter structures, such as the corpus callosum, were identified through tracing the formation of anisotropic regions on the FA maps. The cortex displayed a high level of anisotropy normal to the cortical surface, unlike an adult brain in which the cortex shows isotropic diffusion, indicating possibly the presence of radial glial fibres.

Conclusions: Structural heterogeneities at the boundary between cortical and sub-cortical regions could arise prior to the development of a fold. Whether these 'nodes' are predetermined by signals arising from the germinal epithelium (the origin of cells occupying the cortical mantle), by differential growth of cortical segments determined by the arrival of afferent fibres from thalamic nuclei, or by uneven occupation of cortical segments by tangentially migrating interneurons, remains to be determined.

Geng G et al (2008) J. Med Image Analysis (in review).

USE OF MATHEMATICAL MODELLING TO PREDICT PREGNANCY OUTCOMES AND BEHAVIOUR OF THE UTERUS

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Background: Human pregnancy has several unusual features including the absence of a fall in circulating maternal progesterone concentrations prior to the onset of labour and an exponential rise in circulating maternal corticotrophin releasing hormone peaking at the time of labour. These features make it difficult to extrapolate easily from results in animal models to predict the behaviour of the human uterus during pregnancy. One way of developing an understanding of the behaviour of the human uterus is through mathematical modeling approaches. We have begun to approach this problem using both top down and bottom up modeling techniques.

Hypothesis: That mathematical modeling can predict the behaviour of the human uterus.

Methods: For Top Down Modelling data was accumulated on 500 women followed prospectively through pregnancy with monthly blood sampling and four ultrasounds prior to term. Samples were assayed for CRH, progesterone, estradiol, estriol, alpha fetaoprotein, placental alkaline phosphatase and human chorionic gonadotrophin. Results were analysed with multiple logarithmic regression analysis. For bottom up modeling, data from the literature and from our own studies was used to generate an *in silico* model of the human uterus.

Results: Top Down modeling allowed the correct identification of 92% of women into preterm and term delivery categories. Bottom Up modeling produced results consistent with NFkB playing a major role in infection related preterm delivery and identified the potential for successful prevention with high dose progesterone therapy.

Conclusions: Mathematical modeling is an alternative approach to understanding biological systems when animal experiments are of limited value. Application of this approach to human pregnancy may allow the development of new diagnostic and therapeutic approaches for the prevention of preterm birth.

DEVELOPING A NOVEL ANIMAL MODEL FOR STUDYING THE POSTNATAL EFFECTS OF NEONATAL BRONCHOPULMONARY DYSPLASIA: PRELIMINARY OBSERVATIONS

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Background:: An animal model of neonatal lung injury at the equivalent age babies become viable (~23w human gestation equivalent to 110 DGA sheep), is required to trial therapies which may ameliorate symptoms of chronic lung disease. Premature birth affects up to 10% of all babies and is the most common cause of death and illness in newborn infants.

Aim: To develop and characterise a model of CLD that persists to term and can be studied into adulthood in sheep.

Methods*:* Fetal sheep at 110 days or 125 days of gestational age (DGA) were anaesthetised and underwent surgery to expose the head and neck. The trachea was intubated and the fetuses ventilated in surgery for two hours (Vt= 5ml/kg, PEEP=0mmHg; PIP=40mmHg). The fetuses were chronically catheterised to monitor blood gases and to collect blood for cortisol and cytokine analyses. An amniotic catheter was implanted so that labour could be monitored. Broncho-alveolar lavage was performed at the end of the ventilation period to measure inflammatory cell production. In the first series of experiments, fetuses will be killed at ~140 DGA; in the second series of experiments, lambs will be delivered naturally and their lung function studied for up to six months of age. To examine the effects of ventilation on the lungs, histological and immunohistochemical analyses will be performed at 140 DGA and 6 months after birth.

Results: To date, six singleton fetuses in the first series of studies have been ventilated; of these four were born prior to 140d GA. The plasma cortisol concentrations of fetuses that delivered prematurely were significantly higher than those that continued to 140 DGA. Fetal blood gases were altered during the two hour ventilatory period but were normal after surgery and for the rest of the experiment in all fetuses.

Conclusions: Our preliminary results indicate that fetuses can be ventilated in the surgery while *in utero* and can proceed to 140 DGA. To prevent the frequent preterm deliveries, apparently caused by elevated fetal cortisol levels following ventilation, we may need to treat ewes chronically with progesterone after surgery. Future surgeries will use twins, with one fetus undergoing ventilation and its twin to be used as a control. Both fetuses will be catheterised to monitor blood gases, and perform cortisol and cytokine analysis.

THE GENOMELAB™ GEXP GENETIC ANALYSIS SYSTEM: ANALYSING MULTIPLE GENES IN ONE SAMPLE

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Background: Real time PCR is a method used to quantitate gene expression. However it is limited in its use due to its inability to quantitate more than two genes at once. Recently Beckman Coulter have developed a new machine, the CEQ™ 8000, which has a number of modules including the GenomeLab™ GeXP genetic analysis system. The GenomeLab™ GeXP genetic analysis system has been validated to quantitatively measure up to 30 genes within one sample. The CEQ™ 8000 is a new piece of equipment purchased in late 2007 for the School of Biomedical Sciences within the University of Queensland. This talk will describe the underlying fundamentals and validation of gene expression using the GenomeLab™ GeXP genetic analysis module of the CEQ™ 8000.

Aims/Hypothesis: The wider aim of this project is to determine the genes involved in rat kidney development and function; genes of interest are those involved in the renal renin-angiotensin system, branching morphogenesis/nephrogenesis, apoptosis and fibrosis as well as genes for various sodium channels and aquaporins. The immediate aim is to validate the GenomeLab™ GeXP genetic analysis system for use in rat kidney samples.

Methods: To use the GenomeLab™ GeXP genetic analysis module mRNA samples first undergo reverse transcription (RT) with reverse primers to produce cDNA followed by amplification of the cDNA with PCR using forward primers. Each primer pair is designed so that the gene fragment generated during RT is of a known, specific length. The reverse and forward primers used during RT/PCR are chimeric primers; they have a gene specific component and a universal component. The gene specific component is used in the initial rounds of RT/PCR, during which time the universal components are incorporated into the genes of interest. The universal component is then used in the latter stages of RT/PCR, ensuring that all genes are treated as the same chemical species. Ie., there is no preferential transcription or amplification of a single gene. In this way the gene ratios within a sample are maintained. During PCR a fluorescent dye is incorporated into the sample. Following PCR the samples are combined with DNA size standards (containing a second fluorescent dye) and ran through a linear polyacrylamide denaturing gel in the CEQ™ 8000 during which stage the gene fragments separate according to size. The amount of each gene within a sample is determined via the strength of its fluorescence.

Conclusions: This new technique for the analysis of multiple genes within one sample will allow us to substantially decrease the costs involved in gene analysis.

SUSTAINED HYPERGLYCAEMIA IN THE SHEEP – A NOVEL MODEL TO TEST β-CELL PLASTICITY

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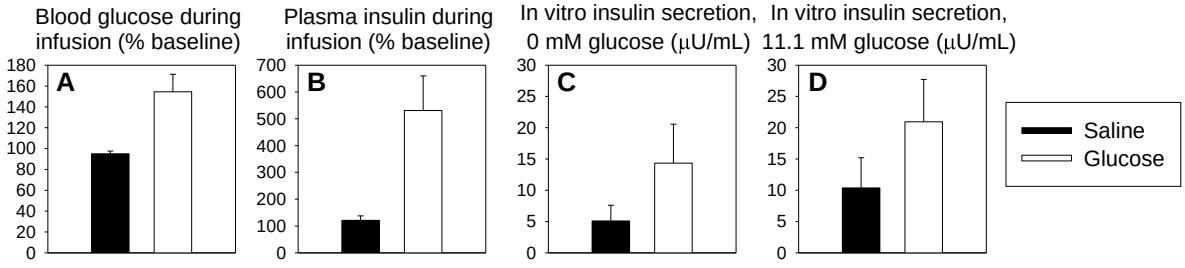
Background: Intrauterine growth restriction and low birth weight in humans increase risk of later diabetes, due to insulin resistance and failure of insulin secretion to adapt and increase to compensate for this (1). We have now shown that this occurs in the placentally restricted and IUGR young adult male sheep, where β-cell insulin secretory capacity is substantially reduced and with onset of insulin resistance, impairs glucose tolerance (2). The PR and IUGR adult male sheep does upregulate β-cell mass, but not enough to compensate for the functional failure (3). We therefore suggest that PR and IUGR impair the capacity to upregulate to adapt β-cell function and mass in response to demand, termed 'plasticity'. Testing this requires a quantitative 'plasticity' challenge. Previous studies have been in rodents using hyperglycaemia and were short term, hence mainly challenged β-cell mass.

Aims: To be able to determine the impact of IUGR on functional plasticity of β-cell function as well as mass and their mechanistic basis, we aimed to develop a model of sustained elevated and controlled demand for insulin in the young sheep.

Methods: Circulating glucose and insulin concentrations were measured before and during a 16-day or 28-day infusion of saline or glucose (25% dextrose). The glucose infusion rate was adjusted daily to achieve a hyperglycaemic clamp and a 50% increase in blood glucose. Saline was infused at comparable rates in controls. Animals were euthanased after 16-days of infusion and pancreas collected for morphometric analysis and islet isolation for *in vitro* study.

Results: Glucose infusion increased circulating glucose (Fig A, +54%) and insulin (Fig B, +431%) relative to pre-infusion levels (P=0.006 and P=0.010 respectively), whilst these did not change in response to saline infusion. In preliminary *in vitro* experiments, chronic hyperglycaemia increased basal (Fig C, +180%) and glucose-stimulated (Fig D, +140%) insulin secretion by isolated islets. Total energy intake (feed plus glucose) was not decreased in response to chronic hyperglycaemia (P>0.05).



Conclusions: Adolescent sheep tolerate a 16-day period of sustained hyperglycaemia well, with no decrease in energy intake. Chronic hyperglycaemic clamp in the sheep substantially upregulates β-cell function *in vivo* in absolute terms (+431%) and *in vitro* (+140%). This establishes the first long term quantitative challenge to β-cell plasticity in any species and suggests that the sheep like the human may rely on substantial upregulation of function in β-cell adaptation to increased demand.

(1) Newsome CA, Shiell AW, Fall CHD, et al. 2003 *Diabet Med* **20**:339-48.
(2) Owens JA, Thavaneswaran P, De Blasio MJ, et al. 2007 Am J Physiol **292**: E1879-89.
(3) Gatford KL, Mohammad SNB, Harland ML, et al. 2008 Endocrinology (in press)

NOTES

PRE-TERM BIRTH: EFFECTS ON NEPHROGENESIS IN THE HUMAN KIDNEY

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Background: Nephrogenesis in the human kidney is complete by 36 weeks of gestation with 60% of nephrons formed in the last trimester; hence it is important to determine the effects of pre-term birth on nephrogenesis.

Aim: To determine the effects of pre-term birth on the number of glomerular generations formed in the pre-term human kidney.

Methods: Archived renal autopsy tissue (embedded in paraffin) dating from 1997 – 2007 of deceased pre-term and term neonates with consent for research were obtained from the Women's and Children's Hospital in North Adelaide. Pre-term neonates (with gestational age ranging from 24 – 34 weeks) that survived postnatally (for 2 – 68 days) with no evidence of growth restriction were selected. Neonates were divided into two groups, the preterm ≤ 30 days group that survived for less than or equal to 30 days postnatally (n=12) and the preterm > 30 days group that survived for greater than 30 days postnatally (n=5). Stillborn neonates delivered at varying gestational ages (20 – 39 weeks) (with an undetermined cause of death) were used for comparison. Paraffin sections stained with haematoxylin and eosin were morphologically assessed and a medullary ray glomerular counting method was utilised to assess the number of glomerular generations formed within the kidney.

Results: Nephrogenesis was on-going in the pre-term ≤ 30 days group (as indicated by the presence of the nephrogenic zone), while nephrogenesis was observed to be complete in the pre-term >30 days group (as indicated by the absence of the nephrogenic zone). In the gestational controls, the number of glomerular generations correlated significantly with gestational age (R^2=0.762, P<0.0001), birth weight (R^2=0.761, P<0.0001) and kidney weight (R^2=0.725, P<0.0001). There were 6.7 ± 0.4 glomerular generations in the pre-term ≤ 30 days group and 8.0 ± 0.4 generations in the pre-term >30 days group, which were not significantly different to the number of glomerular generations in the respective gestational-age-matched controls. Glomerular generations correlated significantly with post-conceptional age (R^2=0.712, P=0.0006) and kidney weight (R^2=0.578, P=0.004) only in the preterm ≤ 30 days group. Abnormal renal morphology was evident in some kidneys. These kidneys contained glomeruli with a dilated Bowman's capsule and also dilated tubules.

Conclusion: The findings to date in this on-going study, suggest that the number of glomerular generations is not reduced in prematurely born neonates. However, abnormalities in renal morphology observed in some pre-term kidneys are likely to adversely impact on renal function.

CROSS SPECTRAL ANALYSIS AND THE QUANTIFICATION OF CEREBRAL AUTOREGULATION IN PRETERM INFANTS

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Background: In recent years, neonatal research has been advanced through the application of engineering methods to clinical problems. Of particular interest is in research of cerebral autoregulation in the preterm brain.
Failure of cerebral autoregulation is a key pathogenic factor in cerebral injury in preterm infants. Traditional assessment of static cerebral autoregulation used time-domain correlation of cerebral blood flow (CBF) and mean arterial blood pressure (MABP). Spatially Resolved Spectroscopy (SRS) continuously measures cerebral tissue saturation (TOI) which reflects changes in CBF. The engineering method of cross spectral analysis may be applied to assess frequency-domain correlation between continuous measurements of TOI and MABP, and determine dynamic cerebral autoregulation in specific frequency ranges.

Aim: To determine the optimal parameters to employ in cross spectral analysis, for quantification of the frequency-dependent covariation of TOI and MABP as a means of assessing dynamic autoregulation

Method: Evaluation was conducted with data obtained from very preterm infants undergoing intensive care during the first 3 postnatal days. TOI was continuously assessed using SRS (Hamamatsu NIRO 200, Japan). MABP was continuously measured via intra-arterial catheter. TOI and MABP were collected at sampling frequency of 6 Hz. Cross spectral analysis (coherence, Coh, MatLab, MA, USA) was used to assess the concordance between TOI and MABP in the frequency domain. At a given frequency, Coh ≥ 0.5 indicates a significant correlation between the MABP-TOI waveforms and therefore impaired autoregulation. Data epochs of 10-, 20- and 30-minute durations were transformed into power spectral densities (PSDs) using different Fourier Transform lengths (nFT), to evaluate the effect on spectral resolution and average Coh across a frequency range.

Results: Data epochs of 30-minutes duration and nFT of 5400 yielded optimal spectral resolution and allowed interrogation of low frequency changes down to 0.001Hz. Variance of the Coh calculation was reduced with division of the epoch into 5 equal length segments overlapped by 75% for transformation into PSDs and averaging.

Conclusions: The selection of appropriate cross spectral analysis parameters is necessary to detect the frequency range of interest, to optimise spectral resolution and to maximise the statistical reliability of the Coh calculation. The practical implication of this is that greater information is made available for assessment of autoregulatory capacity. The use of cross spectral analysis offers a powerful means to quantify cerebral autoregulation in preterm infants.

EFFECT OF INDOMETHACIN, IBUPROFEN AND GENTAMICIN ON GLOMERULAR NUMBER IN A NEONATAL RAT MODEL

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Background: Premature neonates are frequently administered indomethacin, ibuprofen and gentamicin during a period of active glomerulogenesis. These drugs are known to have nephrotoxic effects, but the morphological effect of these drugs is largely unknown.

Aim: To determine whether administration of these drugs during glomerulogenesis has an effect on glomerular, and hence nephron, number in a neonatal rat model.

Methods: Rat pups were exposed to no treatment or intraperitoneal saline (control group), intraperitoneal indomethacin 0.2 mg/kg, ibuprofen 10 mg/kg or indomethacin 0.1 mg/kg and gentamicin 2.5 mg/kg for 5 days postnatally from day 1 of life. The pups were sacrificed at 14 days of age at completion of glomerulogenesis. Following fixation, the total number of glomeruli were counted in the left kidney using the physical disector/fractionator combination technique, an unbiased stereological method.

Results: 6 rat pup kidneys were assessed from each group with equal number of male and female gender and similar weight. Analysis so far of three rat pups from each group showed a mean number of glomeruli in the control group of 28425 (range 23494-31068), indomethacin group 23915 (range 21404-27541), ibuprofen group 27466 (range 24351-31092) and indomethacin and gentamicin group 30490 (range 26764-33418).

Conclusions: There is no statistically significant decrease in glomeruli number following administration of indomethacin, ibuprofen or indomethacin and gentamicin in this neonatal rat model, although there appears to be a trend towards a decreased number in the indomethacin treated group.

SPORIDESMIN-INDUCED MATERNAL LIVER DAMAGE PRIOR TO CONCEPTION ALTERS FETAL ORGAN GROWTH AND PLACENTAL MORPHOLOGY IN THE LATE GESTATION SHEEP FETUS

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Background: Superoxide free radicals produced during oxidation of the mycotoxin sporidesmin, which causes facial eczema in sheep, can cause severe liver damage. During pregnancy we have observed that facial eczema disease can result in cholestatic-like pathology in the mother, severe fetal and placental growth restriction and abortion.

Aim: To determine whether a reproducible experimental model of fetal growth restriction could be developed using controlled exposure to sporidesmin prior to mating.

Methods: Five year old Romney sheep (n = 70) were dosed with sporidesmin extracts until their plasma gamma-glutamyletransferase (GGT) activity was between 250-1000 U/L (normal = 60 U/L). Sheep that met this criterion (SP, n=44) and 42 non-exposed sheep (CN) were then time mated. Pregnant ewes were ethanased at 89d (SP, 9 single, 6 twin; CN, 5 single, 10 twin) and 134d (SP, 8 single, 7 twin; CN, 8 single, 10 twin) and postmortems performed. Data are expressed as regression coefficients and 95% confidence intervals.

Results: With each 100 U/L increase in plasma GGT at mating visual liver damage score (range 1 - 5) in ewes increased at 89d (0.29 $^{0.18,\ 0.41}$) and 134d (0.26 $^{0.2,\ 0.32}$, both $p<0.0001$). Fetal and placental weight and fetal length were not influenced by plasma GGT at mating. At 89d fetal pancreatic weight decreased 21mg ($^{38,\ 4}$, $p<0.05$) with each 100U/L of GGT at mating, while at 134d fetal pancreas (-132mg $^{-221,\ -42}$), spleen (-139mg $^{-231,\ -47}$) and thyroid (-29mg $^{-48,\ -10}$) weight all decreased (all $p<0.01$). At 134d the number of A type placentomes decreased (-3 $^{-5,\ -1}$) while the number of C (+1.3 $^{0.2,\ 2.4}$) and D type (+1.9 $^{1.0,\ 2.8}$) placentomes increased with each 100 U/L of GGT at mating (all $p<0.05$).

Conclusions: Sporidesmin-induced maternal liver damage before mating had little effect on gross fetal and placental size and hence did not provide a useful experimental model of fetal growth restriction. However the effect of pre-mating liver damage on some fetal organs, in particular the pancreas, and placental morphology may suggest that important aspects of fetal nutrient supply have been affected. In both sheep and human pregnancy the effects of moderately impaired maternal liver function may not be readily detected but could still be important for fetal and placental functional development.

PLACENTAL RESTRICTION AND SMALL SIZE AT BIRTH IN SHEEP; CHANGES IN PANCREATIC GENE EXPRESSION EXPLAIN LOSS OF β-CELL FUNCTION AND UP-REGULATION OF β-CELL MASS

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Background: Intrauterine growth restriction and low birth weight in humans increase risk of later diabetes, due to insulin resistance and the failure of insulin secretion to adapt and increase to compensate for this (1). We have now shown that this occurs in the placentally restricted and IUGR young adult male sheep, where β-cell insulin secretory capacity is substantially reduced and with onset of insulin resistance, impairs glucose tolerance (2). The PR and IUGR adult male sheep does upregulate β–cell mass, but not enough to compensate for the functional failure (3). Little is known about the molecular mechanisms underlying fetal programming of β-cell function and β-cell mass following IUGR, particularly in species where pancreatic development occurs predominantly before birth, as in humans.

Aims: To determine the effect of poor growth before birth, induced by restriction of placental growth and function, on expression of molecular determinants of β-cell function and mass in the lamb, and relate gene expression to *in vivo* functional measures in the same animals.

Methods: *In vivo* glucose tolerance, insulin secretion and sensitivity were measured as previously described (4). Pancreas was collected following euthanasia at 43 d of age. Gene expression was measured by qPCR using primers we designed and validated for use in sheep. Embedded pancreas was sectioned, immunostained for insulin positive cells, and point-counted to obtain the volume density of β-cells. β-cell mass was calculated as V_d x pancreas mass.

Results: In the young lamb, PR increased expression of genes that up-regulate β-cell mass, including Pdx-1 (P=0.093), IGF-II (P=0.025) and the insulin receptor (P=0.053). Also in males, expression of IGF-II and the insulin receptor were consistently positively related with β-cell mass (Figure 1). PR also increased expression of Slc2a2 (GLUT2, P=0.083), but substantially decreased expression of the α1D subunit of the voltage-gated calcium channel (P=0.005). In males, the latter was strongly and positively related to glucose-stimulated insulin secretion (Figure 2).

Figure 1

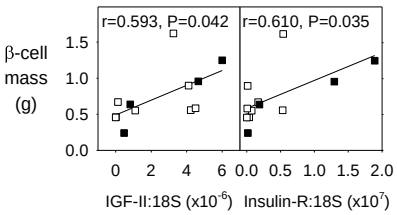
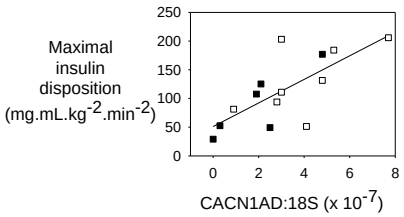


Figure 2



Conclusions: Poor growth before birth in the sheep leads to progressive loss of β-cell function postnatally, particularly in males. We have identified the voltage-gated calcium channel as a novel molecular target for programming of impaired β-cell function by an adverse intrauterine environment. Up-regulation of Pdx-1, IGF-II and insulin receptor gene expression may contribute to the maintenance of insulin secretion relative to sensitivity at this age, with failure before adulthood in males.

(1) Newsome CA, Shiell AW, Fall CHD, et al. 2003 *Diabet Med* **20**:339-48.
(2) Owens JA, Thavaneswaran P, De Blasio MJ, et al. 2007 *Am J Physiol* **292**: E1879-89.
(3) Gatford KL, Mohammad SNB, Harland ML, et al. 2008 *Endocrinology* (in press)
(4) De Blasio MJ, Gatford KL, McMillen IC, et al. 2007 *Endocrinology* **148** : 1350-8.

RAPID POSTNATAL GROWTH IS ASSOCIATED WITH LATER REDUCED GLUCOSE TOLERANCE IN PRETERM BUT NOT TERM LAMBS

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Background It has been suggested that preterm birth may be associated with metabolic derangements later in life similar to those seen in term babies that are small for gestational age. The optimal pattern of postnatal growth after preterm birth has not been defined. Rapid growth after birth is associated with improved neurodevelopmental outcomes, but also with later insulin resistance. It is not clear if the rapid growth *per se* is responsible for the metabolic derangement, or if the postnatal growth pattern is determined by another factor which also affects later metabolism.

Aims To assess the effects of preterm birth, birth weight, nutrient supplementation and neonatal growth on glucose tolerance at 4 months of age.

Methods Romney ewes either lambed spontaneously at term (term lambs, n=13; gestation=147 d) or lambed preterm following dexamethasone-induced labour (preterm lambs, n=21; gestation=137 d). Lambs were randomised to natural suckling only or suckling + nutritional supplements (24kcal, 4g protein per 100ml) for the first 2 weeks of life. Lambs were weighed daily and exponential growth velocity (GV) calculated. Intravenous glucose tolerance tests were performed at 4 months of age and area under the curve was calculated. Data are expressed as regression coefficients and 95% confidence intervals**.**

Results In preterm lambs, lower birth weight was associated with more rapid growth in the first 2 weeks of life (-9.5 (-14,-5); r=0.7, p=0.0002) and up to 4 months of age (-1.9 (-2.7,-1.2); r=0.8, p<0.0001). In preterm lambs, lower birth weight (-163 (-322,-5); r=0.44, p=0.04) and greater percentage weight gain to four months (83 (24,143); r=0.56, p=0.008) were both associated with reduced glucose tolerance at four months, with a stronger effect seen in females. These relationships were not seen in term lambs

Table.1 Growth parameters and glucose tolerance in supplemented and unsupplemented term and preterm twin lambs

	Preterm n=21		Term n=13	
	Supp n=11	Unsupp n=10	Supp n=7	Unsupp n=6
Birth Weight (kg)***	4.0±0.1	4.1±0.1	5.0±0.3	5.6±0.2
GV 1st 2weeks ($g.kg^{-1}.day^{-1}$)	44±2	43±2	47±2†	41±1
Weight at 4 month (kg)	30±0.9	30±0.7	29±1.4	28±1.7
AUC ($mmol.min.L^{-1}$)	678±51	696±48	642±31	620±47

*** p<0.001 for difference between term and preterm
† p<0.05 for difference between supplemented and un-supplemented lambs of the same gestation

Conclusions Lower birthweight is associated with rapid early postnatal growth. However, both birthweight and rapid postnatal growth were associated with reduced glucose tolerance only in preterm lambs. It is likely that both intrauterine and postnatal nutrition contribute to metabolic outcomes in preterm lambs. Manipulation of postnatal nutrition may be one way to ameliorate the effects of an *in utero* insult on later metabolic outcomes.

NOTES

THE EFFECT OF VENTILATOR GAS FLOW RATES ON MARKERS OF ACUTE LUNG INJURY IN PRETERM LAMBS

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Background: Neonatal ventilator gas flow rates are usually set at 8-10 L/min, irrespective of birth weight. We previously have demonstrated in preterm lambs that lower flow rates result in longer inspiratory time and better ventilator efficiency index. Flow rates higher than necessary for efficient ventilation may cause shear stress injury to the immature lung, thereby contributing to ventilator-induced lung injury. Certain connective tissue-related genes have been identified that are up-regulated very early in lung injury; mRNA levels of these genes have been used as markers of early acute lung injury.

Aims: To determine the effects of ventilator gas flow rate on mRNA levels of the early lung injury markers connective tissue growth factor (*CTGF*), early growth response 1 (*EGR1*) and cysteine-rich 61 (*CYR61*) in preterm lambs.

Methods: Following antenatal glucocorticoids to the ewe, 37 lambs (131-133 d gestation, term=147 d) were ventilated for 2 hr (Dräger Babylog 8000*Plus* PSV+VG, tidal volume 7 ml/Kg, PEEP 6 cmH_2O, rate 40 breaths/min) after randomisation to low (L), medium (M) or high (H) flow (8, 18 or 28 L/min). FiO_2 was altered to maintain oxygen saturation >90%. Lung tissue was collected at 2 hours and rapidly frozen, before extracting RNA using a modified guanidine thiocyanate method. Lung *CTGF*, *EGR1, CYR61* and *18S* (housekeeping gene) mRNA levels were measured using quantitative RT-PCR (with SYBR Green as fluorescent dye). mRNA levels were expressed as fold change relative to mean levels of mRNA in age-matched controls. Data were analysed by one-way ANOVA with Fisher posthoc LSD. Significance was taken as $P<0.05$.

Results: *CTGF*, *EGR1* and *CYR61* mRNA levels were increased after ventilation in all animals when compared to controls ($P<0.05$), and *CTGF* and *EGR1* mRNA levels were lower in L than in M lambs ($P<0.05$).

	Control (n=8)	L (n=11)	M (n=12)	H (n=14)
CTGF	1 (0.26) ^{a}	3.9 (0.88) ^{b}	8.1 (1.23) ^{c}	5.3 (1.11) ^{b,c}
EGR1	1 (0.23) ^{a}	5.7 (1.07) ^{b}	10.9 (1.21) ^{c}	7.9 (1.57) ^{b,c}
CYR61	1 (0.22) ^{a}	8.2 (2.3) ^{b}	11.0 (1.12) ^{b}	9.2 (2.10) ^{b,c}

Table 1: mRNA levels for *CTGF*, *EGR1* and *CYR61* expressed as fold change of controls. All values are reported as mean (SEM). Values that do not share a common letter superscript are significantly different from each other ($p<0.05$).

Conclusions: Early response genes *CTGF*, *EGR1* and *CYR61* are markedly upregulated after ventilation, but the upregulation is less in lambs ventilated at a low gas flow rate (~1.7 L/Kg.min). In preterm babies, flow rates as high as 20 L/Kg.min are routinely used. The possibility that high ventilator gas flow rates may contribute to ventilator-induced lung injury via shear stress injury needs further investigation.

RESPIRATORY INSTABILITY: HYPERVENTILATION AND HYPOXIA IN THE LAMB AND HUMAN INFANT VERSUS THE ADULT

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Background: Periodic breathing (PB) is elicited by hypoxia in adults, yet surprisingly PB is not seen during hypoxia in the human preterm infant. Our earlier studies show that hypoxia also does not elicit PB in the lamb. Interestingly, when the lamb and the human infant return to air after a period of breathing hypoxic gas, both exhibit PB. There is a body of evidence that suggests the sensitivity of the carotid bodies changes from additive to multiplicative with increasing postnatal age in the human and some animal species and that this change could explain these different responses to hypoxia and hyperventilation. However, to date there has been no definitive evidence demonstrating that the controller changes in any major way with age.

Aim: To determine whether an additive or multiplicative controller can account for the differing effects of hypoxia on respiratory instabilities.

Methods: We examined the impact of administration of hyperventilation and application of hypoxic gas during PB in a lamb model. Furthermore, we derived a mathematical model of respiratory control in the lamb and used an existing model of the human adult (Khoo et al. 1982), that we have used to elucidate key differences in the causes of instabilities in infants and adults.

Results: Application of hypoxic gas results in an additive ventilatory response in the lamb. A similar additive response has been found in the human infant (Sovik & Lossius, 2004). In adults, hypoxia augments the peripheral response to CO_2 (multiplicative controller), and increases controller gain sufficiently to precipitate PB. In lambs, by contrast, hypoxia does not augment the peripheral response to CO_2, and PB does not occur. Administration of hypoxic gas stopped PB in the lamb. By contrast, in the adult model hypoxia exacerbated PB.

Conclusion: A fruitful approach to determining the likely cause of PB is to focus on the factors that define controller types. Substantial evidence suggests that the respiratory control system of the newborn infant is initially additive and becomes multiplicative with age. Whether a transition in controller from additive to multiplicative confers benefit is not known, but this work has implications for the differing treatment of respiratory instabilities in the newborn infant compared to that for the adult.

AIRWAY REMODELING IN A MODEL OF VENTILATOR-INDUCED INJURY OF THE VERY IMMATURE LUNG

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Background: Very preterm babies usually require ventilatory support, which can lead to ventilator-induced lung injury (VILI) and bronchopulmonary dysplasia (BPD). The long term respiratory effects of very low and extremely low birth weight (VLBW; ELBW) include reduced lung function and an increased risk of asthma in children and adolescents. It is possible that ventilating the very preterm lung causes changes in the conducting airways that could increase the risk of later airway dysfunction. However, little is known about the effects of ventilating the immature lung on the conducting airways.

Aim/Hypothesis: To investigate the effects of mechanical ventilation *per se* on the airways of the very immature lung using a model of *in-utero* ventilation (IUV), and to determine the influence of the duration of ventilation. We hypothesized that mechanical ventilation of the immature lung would adversely alter the structure of the conducting airways.

Methods: Fetal sheep were mechanically ventilated *in-utero* via chronically implanted tracheal catheters at 0.75 of term. One group of fetuses underwent a 6hr IUV period followed by 6hrs of recovery during which no artificial ventilation occurred; the lungs were collected after this recovery period (n=5). In another group, lung tissue was collected immediately following a 12hr IUV period (n=5). A third group underwent a 12hr IUV period followed by a 7d period of recovery, after which the lungs were collected (n=5). Age-matched unventilated fetuses (n=5 per age group) were used as controls. The structure of small conducting airways was morphometrically analyzed.

Results: Following 6hrs of IUV, the airways had an increased amount of smooth muscle and fewer alveolar attachment sites. Immediately following 12hrs of IUV, the airways had an increased amount of smooth muscle, fewer alveolar attachment sites and less collagen deposition; the airway epithelium had increased numbers of mucin containing cells and apoptotic cells, and decreased numbers of proliferating cells. Seven days later the effects of 12hrs of IUV included thickening of the epithelium, increased amounts of smooth muscle and collagen deposition, fewer alveolar attachment sites, and increased epithelial cell proliferation and apoptosis.

Conclusions: Mechanical ventilation of the very immature lung for as little as 6 or 12 hours induces structural alterations in the conducting airways, some of which last for at least 7 days. These changes could be due to inflammation or oxidative injury and could contribute to later airway dysfunction.

IMPACT OF POSTNATAL INCREASE IN OXYGEN SENSITIVITY ON RESPIRATORY INSTABILITY: THEORY

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Background: Periodic breathing (PB) is common in normal term and preterm infants, and occurs in normal adult subjects at high altitude as well as in patients with a variety of conditions including idiopathic central sleep apnoea and congestive heart failure. The reasons for the appearance of PB after the first few days of life and its gradual decline over the following six months remain poorly understood. However, experimental and mathematical modelling studies of the respiratory control system implicate maturation of carotid body chemoreception in the genesis of PB.

Hypothesis: The postnatal increase in oxygen sensitivity known to occur in the first months of life decreases the stability of the respiratory control system and leads to the appearance of PB.

Methods: Using a detailed mathematical model of the respiratory control system of the infant, we investigated the impact of a sigh on ventilatory output and on loop gain (LG) of the system as we increased carotid body oxygen sensitivity. LG was used to explicitly quantify the stability of the control system and to predict the occurrence of instability. Oxygen sensitivity was derived from published data on the magnitude of the response to 15 sec of hyperoxia (Søvik et al. 1999). Factors were altered to further decrease stability (e.g. reduced lung volume and reduced central ventilatory drive) to produce PB.

Results: The postnatal increase in oxygen sensitivity increases LG of the respiratory control system. With an increase in oxygen sensitivity, PB can be produced following a sigh, when lung volume, central ventilatory drive, or cardiac output, are reduced.

Conclusion: The postnatal increase in oxygen sensitivity, despite providing protection of the infant from hypoxaemia, decreases the stability of the respiratory control system and predisposes to periodic breathing, especially when combined with other destabilising features that commonly affect the newborn, such as reduced lung volume and cardiac output.

AKT AND MAPK SIGNALING IN VENTILATED PRETERM LAMB DIAPHRAGMS

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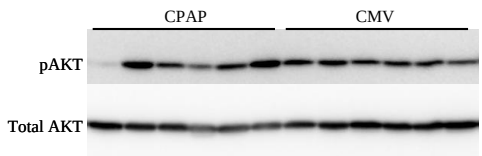
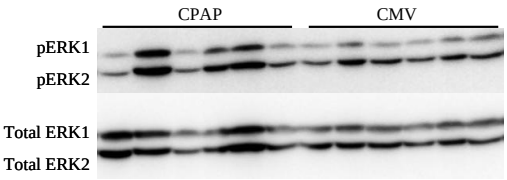
Background: Preterm babies requiring prolonged controlled mechanical ventilation (CMV) can be difficult to wean and extubate successfully. Ventilator Induced Diaphragmatic Dysfunction (VIDD) is a condition characterized by a decrease in the diaphragm's force generating capacity associated with increased muscle atrophy. In muscle cells, the Akt and MAPK signaling pathways regulate events linked to protein metabolism and cell proliferation. Changes in the activity of these pathways may disrupt the balance of protein. Diaphragmatic atrophy has been found in postmortem samples of newborns receiving prolonged CMV. No studies have investigated VIDD in newborns and the acute consequences of ventilation on the signaling pathways in the preterm neonatal diaphragm.

Aims/Hypothesis: We aimed to measure the total and phosphorylated Akt and MAPK (ERK1 and ERK levels in diaphragm muscles after 3 hours of CMV or continuous positive airway pressure (CPAP). We hypothesised that compared to preterm lambs breathing spontaneously on CPAP, preterm lambs managed with CMV will show decreased activity of Akt and MAPK pathways.

Methods: Preterm lambs (133 d, term = 150 d) were delivered by caesarean section and randomized to either CPAP (n=16) or CMV (n=16). CMV lambs were sedated using IV remifentanil and propofol. Lambs were euthanased after 3 h with IV pentobarbitone (100 mg/kg). Diaphragms were collected immediately post-mortem. Phosphorylated and total Akt and MAPK (ERK1 and ERK2) protein levels were measured using western blot assays.

Results: Preliminary results show no changes in phosphorylated or total Akt levels. Phosphorylated and total ERK1 and ERK2 levels appear decreased in the CMV group compared to the CPAP group.





Conclusions: Decreased activation of the MAPK signaling pathway in the CMV group compared to the CPAP may be associated with a negative effect of CMV on transcription and hence impairment of muscle growth and fiber synthesis. Our data suggest that compared to CPAP lambs, the CMV lambs are at increased risk of developing muscle atrophy and subsequent VIDD. Longer periods of ventilation may be required to affect changes in the Akt signaling pathway.

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THE EFFECTS OF ALCOHOL EXPOSURE ON THE GROWTH, MATURATION AND INFLAMMATORY STATE OF THE FETAL LUNG.

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Background: Fetal alcohol exposure is known to affect brain development, but few studies have examined the effects of alcohol on the development of the lung. Chronic alcohol exposure during pregnancy has been shown to cause both body and lung growth restriction in rodents (Inselman et al., 1985) in which lung parenchymal development occurs largely after birth. Alcohol exposure during late gestation, in species such as human and sheep where considerable development of the lung parenchyma occurs *in utero*, may affect alveolar formation and lung maturation, including surfactant production. Previous studies have suggested that surfactant synthesis may be altered by alcohol exposure (Lazic et al., 2007). We are also interested in the effects of alcohol exposure on lung inflammation in the fetus, as alcohol exposure in adult lungs has been shown to cause a decrease in pro-inflammatory cytokines (D'Souza El-Guindy et al., 2007).

Aims/Hypothesis: To determine the effects of daily alcohol exposure during the last third of gestation on the growth, maturation and inflammatory status of the fetal lung. We hypothesized that fetal alcohol exposure would adversely affect the development of the lung.

Methods: Pregnant ewes were chronically catheterized at 91 days of gestational age (d GA; term ~147d). From 95-133d GA, ewes were given a one hour daily infusion of either 0.75g/kg ethanol (n=9) or saline (n=8), with tissue collection occurring at 134d GA. Fetal lung tissue was examined for changes in DNA and protein contents, cell proliferation and elastin content. The mRNA expression of the surfactant proteins (SP)-A, B, C and D and the pro-inflammatory cytokines; IL-1β, IL-6, IL-8 and TNF-α, was examined using real-time PCR.

Results: There were no differences in fetal body weights, lung weights, DNA and protein contents, percent proliferating cells and elastin content between treatment groups. SP-A ($p<0.05$), SP-B ($p<0.05$) and SP-D ($p=0.083$) mRNA expression was reduced to approximately one third of control levels following ethanol exposure. IL-1β and IL-8 mRNA levels were significantly lower ($p<0.05$) in lung tissue from ethanol-exposed fetuses compared to controls and TNF-α mRNA levels tended to be reduced ($p=0.103$).

Conclusions: Daily exposure to a modest amount of alcohol during the last third of gestation does not affect overall lung growth, but the reduction in surfactant protein mRNA levels suggests that fetal alcohol exposure may alter alveolar epithelial cell differentiation. Additionally, by causing a reduction in SP and pro-inflammatory cytokine mRNA levels, fetal alcohol exposure changes the innate immune status of the fetus, which could increase the susceptibility of the lungs to infection.

D'Souza El-Guindy et al (2007) *Alcohol,* 41, 335-345.
Inselman et al (1985) *Pediatr Res,* 19, 12-14.
Lazic et al (2007) *Alcohol,* 41, 347-355.

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