

**THE NINETEENTH NATIONAL WORKSHOP ON FETAL  
AND NEONATAL PHYSIOLOGY**

**A WORKSHOP IN HONOUR OF  
PROFESSOR MARELYN WINTOUR**



**Florey Lecture Theatre, Medical School, University of Adelaide,  
Adelaide, South Australia, March 12-13, 2005**

*Organising Committee:*

*Prof Julie Owens, University of Adelaide*

*Prof Richard Harding, Monash University*

*Dr Megan Probyn, Monash University*



## Program at a glance

Saturday 12 <sup>th</sup>	Sunday 13 <sup>th</sup>
<b>9.30-10.30: Registration/morning tea</b>	
<b>SESSION 1</b>	<b>SESSION 4</b>
10.30-10.45 A1	9.00-9.15 A17
10.45-11.00 A2	9.15-9.30 A18
11.00-11.15 A3	9.30-9.45 A19
11.15-11.30 A4	9.45-10.00 A20
11.30-11.45 A5	10.00-10.15 A21
11.45-12.00 A6	
<b>12.00-12.30: General discussion</b>	<b>10.15-10.40: General discussion</b>
<b>12.30-1.30: Lunch</b>	<b>10.40-11.10: Morning tea</b>
<b>SESSION 2</b>	<b>SESSION 5</b>
1.30-1.50 "M. Wintour & Fetal Physiology"	11.10-11.25 A22
1.50-2.05 A7	11.25-11.40 A23
2.05-2.20 A8	11.40-11.55 A24
2.20-2.35 A9	11.55-12.10 A25
2.35-2.50 A10	12.10-12.25 A26
2.50-3.05 A11	
<b>3.05-3.30: General discussion</b>	<b>12.25-12.50: General discussion</b>
<b>3.30-4.00: Afternoon tea</b>	<b>12.50-1.50: Lunch</b>
<b>SESSION 3</b>	<b>SESSION 6</b>
4.00-4.15 A12	1.50-2.05 A27
4.15-4.30 A13	2.05-2.20 A28
4.30-4.45 A14	2.20-2.35 A29
4.45-5.00 A15	2.35-2.50 A30
5.00-5.15 A16	2.50-3.05 A31
<b>5.15-5.40: General discussion</b>	<b>3.05-3.30: General discussion</b>
	<b>3.30-4.00: Afternoon tea</b>
	<b>SESSION 7</b>
	4.00-4.15 A32
	4.15-4.30 A33
	4.30-4.45 A34
	4.45-5.00 A35
	<b>5.00-5.20: General discussion</b>
<b>5.45: Pre dinner drinks</b>	<b>5.20: Presentation of Student prizes*</b>
<b>7.30: Dinner at University of Adelaide staff club</b>	<b>6.00: PSANZ Welcome Reception</b>

\*Prizes will be awarded to the best student presentation and the best student discussant

## Saturday 12<sup>th</sup> March

### 9.30-10.30 Registration / Morning tea

<b>Session 1 - Placental development: Chair – Prof. Caroline McMillen</b>			
10.30	A1	K.G. Pringle	Role of oxygen and IGF-II on early trophoblast outgrowth and gene expression in the mouse
10.45	A2	A.N. Sferruzzi-Perri	Maternal insulin-like growth factor-II in early pregnancy promotes placental development, fetal growth and viability
11.00	A3	C.T. Roberts	Repeated maternal glucocorticoid treatment in sheep alters placental gene expression
11.15	A4	J.J. Bromfield	Male seminal factors influences fetal and placental development, postnatal growth and adult onset obesity in mice
11.30	A5	S.M. MacLaughlin	Periconceptional undernutrition alters the relationship between maternal body weight and condition and the growth of the placenta and fetus in singleton and twin pregnancies in the sheep during early gestation
11.45	A6	C.J. Fletcher-Hillier	Reproductive cloning alters placental structure in the sheep
12.00-12.30 General discussion			

### 12.30-1.30 Lunch

<b>Session 2 - Prof M Wintour Session: Chair – Prof. Julie Owens</b>			
1.30		J. Owens	Marelyn Wintour – where would fetal physiology be without her?
1.50	A7	E.M. Wintour	Differential effects of early pregnancy treatment with natural and synthetic glucocorticoids in sheep.
2.05	A8	K. Moritz	Prenatal glucocorticoids and the kidney - an update
2.20	A9	C.C. Hoppe	Fetal programming of a nephron deficit in the mouse by maternal protein restriction
2.35	A10	H. Dickinson	Renal function and prenatal programming of hypertension in the spiny mouse
2.50	A11	K.M. Denton	Programming of the renal sympathetic nerves in offspring of hypertensive mothers
3.05 – 3.30 General discussion			

### 3.30 - 4.00 Afternoon tea

<b>Session 3 - Cardiovascular function: Chair – Prof. Adrian Walker</b>			
4.00	A12	K. Suzuki	Effects of fetal lung hypoplasia (LH) on the structure of pulmonary vasculature in sheep
4.15	A13	N. Parange	Changes in fetal ductus arteriosus flow in uteroplacental insufficiency
4.30	A14	C.W.H Rumball	Blood volume parameters of periconceptionally undernourished pregnant ewes
4.45	A15	Y.S Feng	Endotoxin impairs cerebral circulation during REM sleep in new born lambs
5.00	A16	K.J. Bubb	Effects of intrauterine growth restriction on cardiomyocyte maturation and function of small coronary arteries
5.15 – 5.40 General discussion			

5.45 pm: Wine tasting - "Best of South Australia"

7.30 pm: Workshop dinner - University of Adelaide Staff Club

## Sunday 13<sup>th</sup> March

<b>Session 4 - Renal function and development: Chair – Prof. Marelyn Wintour</b>			
9.00	A17	W-M. Boon	Genomic and proteomic applications in kidney development
9.15	A18	L. Rattanaray	Perinatal rofecoxib exposure impairs normal renal development of offspring in adulthood
9.30	A19	R. Singh	Exploring mechanisms through which glucocorticoids affect the growth and development of the kidney
9.45	A20	K.J. Gibson	Net filtration pressure and tubuloglomerular feedback in fetuses and lambs
10.00	A21	A.E. O'Connell	Renal function, arterial pressure and body growth in lambs born to sub-totally nephrectomised mothers
10.15-10.40 General discussion			

### 10.40 - 11.10 Morning tea

<b>Session 5 – Nutrition: Chair – Dr. Frank Bloomfield</b>			
11.10	A22	J.A. Duffield	Gender specific programming of peroxisome-proliferator activated receptor- $\gamma$ and leptin mRNA expression in visceral fat in low birth weight lambs
11.25	A23	B.S. Muhlhausler	The impact of maternal overnutrition in late gestation and gender on fat deposition and feeding behaviour in postnatal lambs
11.40	A24	A.L. Jaquiere	Effect of periconceptional undernutrition on insulin sensitivity at 65 days gestation in singleton bearing ewes
11.55	A25	K. Farrand	Differential effects of gestational age and placental restriction on the proportions of specific corticotroph subpopulations in the fetal sheep pituitary during late gestation
12.10	A26	K. Gaford	Inadequate compensatory increase in beta cell mass and insulin secretion in young adult males following placental restriction of fetal growth in sheep
12.25 – 12.50 General discussion			

### 12.50 - 1.50 Lunch

<b>Session 6 - Development and injury: Chair – A. Prof. Stuart Hooper</b>			
1.50	A27	S. Gentili	Tissue specific expression of Suppressor of Cytokine Signaling (SOCS)-3 during fetal development
2.05	A28	D.A. Todd	Surfactant phospholipid species: changes in endotracheal aspirates of neonates who develop bronchopulmonary dysplasia
2.20	A29	P. Dalitz	Acute maternal alcohol administration can induce white matter injury in the preterm ovine brain
2.35	A30	L. Liu	The effect of antenatal alcohol and fish oil exposure on cardiovascular development in rats
2.50	A31	A.M. Thiel	Expression and localization of IRF2-BP2 in ventilator-induced lung injury
3.05 - 3.30 General discussion			

### 3.30 - 4.00 Afternoon tea

<b>Session 7 – Neonatal: Chair – Prof. Richard Harding</b>			
4.00	A32	M.J. Stark	Characterization of sex-specific differences in preterm neonatal cardiovascular adaptation - a study proposal
4.15	A33	S.R. Yiallourou	Measurement of cardiovascular variables during sleep in infants aged 2-4 months: effects of sleeping position and sleep state
4.30	A34	H.L. Richardson	Variability of the hypoxic ventilatory response in sleeping infants
4.45	A35	S.B. Hooper	Imaging the lungs after birth using a synchrotron
5.00 – 5.20 General discussion			

5.20 pm: Student Prizes (best presentation; best discussant)

6.00-8.00 pm: PSANZ Reception

# A1

## ROLE OF OXYGEN AND IGF-II ON EARLY TROPHOBLAST OUTGROWTH AND GENE EXPRESSION IN THE MOUSE

**Pringle KG**, Kind KL, Thompson JG & Roberts CT

Research Centre for Reproductive Health, Department of Obstetrics and Gynaecology, University of Adelaide, Adelaide, 5005

kirsty.pringle@adelaide.edu.au

**Background:** Early human placentation occurs in a low oxygen environment, where the trophoblasts invade into the maternal tissue and its vasculature to gain an adequate nutrient supply for the fetus. Poor placentation may result in miscarriage, preeclampsia or intrauterine growth restriction. Hypoxia Inducible Factor-1 $\alpha$  is a transcription factor involved in the regulation of Insulin-Like Growth Factor-II (IGF-II), Vascular Endothelial Growth Factor (VEGF) and Glucose Transporter 1 (GLUT-1). All of these molecules are widely expressed in early pregnancy and are critical for both fetal and placental growth. Previous work in our group has shown that low oxygen and exogenous IGF-II promote trophoblast outgrowth and increase IGF-II mRNA expression in human placental villous explant cultures. Therefore, this study aimed to determine the effects of oxygen and exogenous IGF-II on murine ectoplacental cone (EPC: trophoblast stem cells of the early placenta) outgrowth and mRNA expression.

**Method:** C57/Bl6 males were mated with CBA F1 females and the day of the vaginal plug was designated as day 0.5 of pregnancy. Day 7.5 EPCs were dissected and cultured for 3 days in 20%, 5% or 1% O<sub>2</sub> on growth factor reduced Matrigel either with or without the addition of 125ng/ml IGF-II and their area of outgrowth was quantified. Real time transcription RT-PCR was used to quantify murine 18S, IGF-II, IGF-2R, HIF-1 $\alpha$ , VEGF and GLUT-1 mRNA concentrations. Data was analysed by univariate ANOVA with Bonferroni post hoc analysis.

**Results:** EPC outgrowth was reduced by 16.8% following culture in 5% O<sub>2</sub> (p<0.05) and by 34.7% following culture in 1% O<sub>2</sub> (p=0.009), compared with 20% O<sub>2</sub> cultures. IGF-II had no effect on the area of EPC outgrowth. Oxygen significantly affected the EPC mRNA expression of IGF-II (p<0.01), HIF-1 $\alpha$  (p<0.005) and GLUT-1 (p<0.05). IGF2R and VEGF mRNA expression was similar between treatment groups. HIF-1 $\alpha$  mRNA expression was ~25% lower in the 5% O<sub>2</sub> group and 50% lower in the 1% O<sub>2</sub> group than those cultured in 20% O<sub>2</sub>. Conversely, GLUT-1 mRNA expression was increased by 2.5 fold when cultured in 1% O<sub>2</sub> compared to 20% O<sub>2</sub> (p<0.005). Treatment with IGF-II at 1% O<sub>2</sub> decreased GLUT-1 mRNA expression when compared to EPCs grown in 1% O<sub>2</sub> without IGF-II (p<0.05).

**Conclusions:** Our data suggest that low O<sub>2</sub>, but not IGF-II, inhibits EPC outgrowth and decreases IGF2 and HIF-1 $\alpha$  gene expression. Although hypoxic regulation of HIF-1 $\alpha$  is thought to be regulated at the protein level and not at the transcriptional level, there are reports of decreased HIF-1 $\alpha$  mRNA under prolonged hypoxia. Despite a reduction in HIF-1 $\alpha$  mRNA, EPC GLUT-1 mRNA expression increased in response to low O<sub>2</sub>, therefore, further investigation of HIF-1 $\alpha$  protein, and other HIF- $\alpha$  isoforms (HIF-2 $\alpha$  and -3 $\alpha$ ) is required to determine what mediates this response. These results suggest that low O<sub>2</sub> induces a hypoxic-stress response in the EPC, such that EPC outgrowth is reduced and glucose transporters are upregulated. This was unexpected, since low O<sub>2</sub> is essential for early human placentation and is a key determinant of pregnancy success.

## A2

### **MATERNAL INSULIN-LIKE GROWTH FACTOR-II IN EARLY PREGNANCY PROMOTES PLACENTAL DEVELOPMENT, FETAL GROWTH AND VIABILITY**

AN Sferruzzi-Perri<sup>1,2</sup>, JA Owens<sup>2</sup>, JS Robinson<sup>2</sup> and CT Roberts<sup>1,2</sup>

<sup>1</sup>Research Centre for Reproductive Health, <sup>2</sup>Dept of Obstetrics and Gynaecology, University of Adelaide, Adelaide, South Australia.

[amanda.sferruzzi-perri@adelaide.edu.au](mailto:amanda.sferruzzi-perri@adelaide.edu.au)

**Aims:** Insulin-like growth factor (IGF)-I and -II regulate growth and development and are expressed in many tissues in the mother, placenta and fetus. IGF-II, in particular, is essential for placental development especially in early pregnancy. Circulating IGFs in the mother increase during pregnancy in women and guinea pigs and correlate positively with placental and fetal growth, suggesting an important endocrine role for maternal IGFs. Maternal IGF treatment in guinea pigs during early pregnancy increases placental and fetal size by mid-gestation (Sohlstrom *et al.*, 2001; Growth Horm IGF Res. 11:392-8), but whether these anabolic effects persist is unknown. This study aimed to determine the effects of maternal IGF-I and -II supplementation in early pregnancy in the guinea pig on placental structural development, fetal growth and maternal body composition near term.

**Methods:** On day 20 of pregnancy (term~70days), mothers were anaesthetised and a mini-osmotic pump was implanted subcutaneously, to deliver 1mg/kg/day IGF-I (n=7), IGF-II (n=9) or vehicle (n=7) for 18 days. Guinea pigs were killed on day 62 of pregnancy. Fetal and placental weights and maternal and fetal body composition were measured. Using Masson's trichrome stain and double-label immunohistochemistry, placental structure was analysed morphometrically. Data were analysed by ANOVA (SPSS).

**Results:** Total litter size was unaffected by either IGF treatment, however, IGF-II increased the number of viable fetuses by 26% (p=0.034) and IGF-I reduced the number of resorptions by 77% (p=0.009). After adjusting for the number of viable pups per litter, maternal IGF-I and IGF-II treatment increased fetal growth by 17% and 11%, respectively (p<0.04), and increased fetal triceps weight in absolute and relative to body weight terms (p<0.03). In contrast, IGF-I treatment reduced fetal liver, brain and spleen weights relative to body weight (p<0.004). IGF treatment did not alter placental weight, but IGF-II increased the cross-sectional area of the exchange region by 28% (p=0.054), the proportion of placenta involved in exchange by 8% (p=0.006) and the volume of the placental exchange region by 27% (p<0.04). The total surface area of trophoblast for exchange was also increased by 39% following IGF-II treatment (p=0.037). Within the exchange region of the placenta, IGF-II treatment increased the volume of trophoblast by 29% (p=0.015) and maternal blood spaces by 46% (p=0.002). In contrast to IGF-II, IGF-I treatment failed to alter any aspect of placental structure. IGF treatment did not affect maternal weight gain during pregnancy, however IGF-I reduced maternal lung and adipose tissue weights (p<0.05).

**Conclusion:** Maternal IGF-I and -II treatment during early to mid pregnancy increases fetal size and viability near term, possibly via different mechanisms. IGF-II persistently enhanced placental structural determinants of function, which should increase substrate supply to the fetus and growth. In contrast, IGF-I failed to affect the placenta but reduced maternal adiposity, suggesting persistent diversion of substrates from mother to conceptus. Increasing maternal IGF-II abundance in early to mid pregnancy may be a potential therapeutic approach to prevent placental insufficiency.

## A3

### REPEATED MATERNAL GLUCOCORTICOID TREATMENT IN SHEEP ALTERS PLACENTAL GENE EXPRESSION

**Roberts CT<sup>1,2</sup>, Moss TJM<sup>3</sup>, Button JJ<sup>1,2</sup>, Hospers M<sup>1,2</sup>, Nitsos F<sup>3</sup>, Harding R<sup>4</sup>, Newnham JP<sup>3</sup>**

<sup>1</sup>Research Centre for Reproductive Health, University of Adelaide, Departments of Obstetrics & Gynaecology <sup>2</sup>University of Adelaide and <sup>3</sup>University of Western Australia and <sup>4</sup>Dept Physiology, Monash University.

Maternal treatment with antenatal glucocorticoids improves neonatal outcome following preterm birth. In sheep, maternal intramuscular betamethasone injections (Mat; 0.5 mg/kg ewe weight) and direct fetal intramuscular betamethasone injections (Fet; 0.5 mg/kg estimated weight) improve preterm lung function. However, maternal, but not fetal, treatment causes fetal growth restriction suggesting impaired placental function after maternal administration. Our aims were to assess placental structural development and gene expression in sheep following single (1-beta) or repeated (3-beta) maternal or fetal intramuscular injections of betamethasone. Forty-one ewes were injected with 150mg medroxy-progesterone acetate on day 98 of pregnancy (term is 150 days) and randomised to maternal or fetal saline (104, 111, 118 days), 1-beta (104 days, followed by saline at 111, 118 days) or 3-beta (104, 111, 118 days) treatment groups. At 146 days, ewes were killed and fetuses and all placentomes weighed. Each placentome was classified according to shape (types A-D), fixed and processed for image analysis. We have previously reported that fetal weight was reduced in only the Mat 3-beta group (p=0.031) but total placental weight was unaffected. However, the proportion of placentomes which were of the 'immature' A-type was greater in Mat than Fet treatment groups (p=0.022), while that of type B placentomes was increased in the Mat 3-beta group (p=0.006). The area of feto-maternal syncytium (FMS) was increased in Mat vs Fet treatment groups (p=0.05). In order to determine whether the treatments caused sustained changes in placental gene expression that could impact on placental function, RNA was extracted from B or C placentomes from saline and 3-beta maternal and fetal treated groups. *Igf2*, *Igf2r* and *Glut-1* mRNA relative to *18S* rRNA expression was quantified with real time RT-PCR using the standard curve method.

	Mat. saline	Mat. 1-beta	Mat. 3-beta	Fetal saline	Fet. 1-beta	Fet. 3-beta
Fetal Weight (kg)	5.19±0.31	4.8±0.36	4.04±0.2 <sup>‡</sup>	5.44±0.3*	5.34±0.2*	5.15±0.2*
Prop. Type A	0.57±0.16	0.66±.14	0.49±0.13	0.18±0.1*	0.19±0.1*	0.55±0.2*
Prop. Type B	0.04±0.02	0.04±0.02	0.2±0.05 <sup>‡</sup>	0.09±0.06	0.17±0.06	0.13±0.05
Area FMS (µm <sup>2</sup> )	2668±154	3042±764	3321±436	2574±216*	2480±349*	2241±393*
IGF-II mRNA	0.54±.05	nd	0.35±0.02 <sup>‡</sup>	0.74±0.17*	nd	0.58±0.05*
IGF2R mRNA	0.90±0.07	nd	0.67±0.06 <sup>‡</sup>	1.08±0.13*	nd	1.17±0.08*
Glut-1 mRNA	0.72±0.16	nd	0.46±0.10	0.68±0.34	nd	0.31±0.13

\*Different between maternal & fetal treatment p<0.05 <sup>‡</sup>Different between saline & 3-beta p<0.05. nd = not done

These results suggest that repeated maternal betamethasone treatment retards placental structural maturation and induces sustained reductions in placental expression of genes involved in tissue remodelling (IGF-II and IGF2R) and placental transport (IGF-II). Reduced IGF-II may impair the diffusional exchange capacity of the placenta as found in placenta specific P0 IGF-II transcript knockouts(1) in which both passive permeability and active transport of amino acids in late gestation are reduced(2). In addition, reduced IGF-II and IGF2R may impair the remodelling of the placenta that is observed late in gestation (ie placental maturation). The increased fetal overgrowth observed in B-D placentomes is a prime example of cell migration and remodelling. Acute reductions in IGF-II expression following glucocorticoid treatment have previously been reported in a range of other tissues and cell lines. Structural and gene expression studies in placentomes sampled during the final week of treatment are essential to understanding the acute effects of repeated glucocorticoid treatment of the mother on placental structure, gene expression and function.

## **MALE SEMINAL FACTORS INFLUENCES FETAL AND PLACENTAL DEVELOPMENT, POSTNATAL GROWTH AND ADULT ONSET OBESITY IN MICE.**

**John J Bromfield**, Claire T Roberts and Sarah A Robertson

Centre for Reproductive Health, Department of Obstetrics and Gynaecology, University of Adelaide, South Australia, Australia.

The development of an optimal uterine environment early in pregnancy is critical for the future development of the fetus and placenta. Constituents within seminal plasma, derived primarily from the seminal vesicle glands, are known to be powerful modulators of the early uterine environment, through regulating female tract cytokine expression and tissue remodeling. It is unclear however whether the absence of seminal plasma at the time of conception has detrimental effects on the outcomes of pregnancy or the subsequent progeny.

In this paper we investigated the impact of initiating pregnancies in the absence of seminal plasma by means of utilising male mice to which the seminal vesicles had been surgically removed. Seminal vesicle deficient males displayed a highly significant perturbation in their ability to initiate pregnancies when compared to intact males, due primarily to a failure in fertilization potential. Outcomes for viable implantation were also adversely affected. Near term placentas from these matings displayed a 15% increase in weight compared to those from control matings. While fetal and new born weights were significantly reduced, the progeny of seminal vesicle deficient males displayed 'catch-up growth' during puberty. These progeny proceeded to become heavier than control progeny, with male progeny displaying an increased abdominal fat deposition. Male progeny displayed reduced plasma adiponectin and increased levels of plasma leptin, a profile associated with obesity. Female progeny exhibited increased plasma insulin and significantly increased levels of glucose and free fatty acids.

This study is the first to show that uterine exposure to seminal plasma at the time of conception has a major impact on the development of the fetus, placenta and progeny of these pregnancies.

## A5

### **Periconceptual Undernutrition Alters the Relationship Between Maternal Body Weight and Condition and the Growth of the Placenta and Fetus in Singleton and Twin Pregnancies in the Sheep During Early Gestation**

**SM MacLaughlin**<sup>1</sup>, SK Walker<sup>2</sup>, CT Roberts<sup>3</sup> and IC McMillen<sup>1</sup>

<sup>1</sup>Discipline of Physiology, <sup>3</sup>Department of Obstetrics and Gynaecology, University of Adelaide, <sup>2</sup>South Australian Research and Development Institute. Adelaide, Australia

Recent studies have shown that a suboptimal maternal environment during embryo development can alter fetal growth and gestation length and is associated with an increased prevalence of adult hypertension and cardiovascular disease. It also appears that maternal weight, or body condition at the start of pregnancy may play a role in fetoplacental growth in early pregnancy and that this role differs in multifetal and singleton pregnancies. We have previously shown that periconceptual maternal undernutrition reprograms the allocation of embryonic totipotent cells into the inner cell mass and trophectoderm with an increase in the proportion of trophectoderm cells in D 7 blastocysts. We have therefore tested the hypotheses that firstly, there are relationships between maternal weight or body condition at the time of conception and fetoplacental growth during the first 55 days of pregnancy and that secondly, periconceptual undernutrition (PCUN) has differential effect on these relationships in singleton and twin pregnancies. We have investigated the effect of periconceptual undernutrition of the ewe [control n= 24, restricted (70% of control feed allowance, PCUN) n=21] from 45 days prior to mating until 7 days post coitum on the fetal and placental development during the period of maximal placental growth. We have also determined the effect of fetal number (singleton compared to twins) on placental development. There was no effect of treatment or fetal number on individual fetal weight, however, there was an interaction of fetal number and PCUN on ponderal index. In control singleton pregnancies there was positive relationships between change in maternal weight during the periconceptual period and fetal weight and placental weight, but not in PCUN singleton pregnancies. In PCUN twin pregnancies there was negative relationships between maternal weight change before post-mortem and fetal and placental weights, but not in control twin pregnancies. The mean weight of placentomes was greater in twins when compared to singletons ( $4.068\text{g} \pm 0.302$  vs  $2.555\text{g} \pm 0.138$ ) irrespective of nutritional regimen. We conclude that fetal number alters fetal and placental development, where demand on the placenta is presumably greater and that there is an effect of maternal weight and body condition change and PCUN during early pregnancy on fetal and placental development, which is different in singleton and twin pregnancies. These observations support previous work from this laboratory, which has shown an impact of fetal number on fetal development in late gestation and that periconceptual undernutrition in twin pregnancies alters development of the cardiovascular system in fetal sheep. These effects may be mediated by altered placental development.

## A6

### REPRODUCTIVE CLONING ALTERS PLACENTAL STRUCTURE IN THE SHEEP

Hillier CJ<sup>1</sup>, Hartwich K<sup>2</sup>, Walker S<sup>3</sup>, Roberts CT<sup>1</sup>, McMillen IC<sup>2</sup>

<sup>1</sup>Research Centre for Reproductive Health, Department of Obstetrics & Gynaecology University of Adelaide <sup>2</sup>Research Centre for the Early Origins of Adult Health, Discipline of Physiology, School of Molecular & Biomedical Science University of Adelaide <sup>3</sup>South Australian Research Development Institute Turretfield

**Background:** The efficiency of reproductive cloning by somatic cell nuclear transfer in livestock is poor with ~ 5% of transferred cloned embryos developing normally and surviving to term. It has been proposed that fetal abnormalities in clones occur as a consequence of placental abnormalities but there have been relatively few reports on the impact of somatic cell nuclear transfer (NT) on placental microstructure.

**Results:** A total of 209 recipient ewes were used for blastocyst transfer, 44% (n=93) were confirmed pregnant and 23% (n=50) died throughout gestation. Placentae from NT cloned lambs (n=11) and age matched naturally mated controls (n=40) were collected at two gestational age (GA) ranges (105-134d or 135-154d). Structural correlates of placental function were quantified using point and intercept counting.

	Control≤134 (n=22)	Clone≤134 (n=6)	Control≥135 (n=18)	Clone≥135 (n=5)
Total placental weight (g)	601.5±46.6	799.2±172.4	512.4±55.3	386.2±52.7
Placentome number	55.0±4.19	44.7±7.96*	72.2±5.05	36.6±5.08*
Individual placentome weight	10.58±1.33	18.60±2.84*	6.58±0.56	6.95±2.03
Proportion intact trophoblast	0.44±0.02	0.16±0.05*	0.40±0.02	0.11±0.05*
Proportion shed trophoblast	0±0	0.24±0.05*	0±0	0.30±0.04*
Surface area (m <sup>2</sup> ) placentome	2.4±0.6	12.6±3.2*	2.5±0.3	4.9±1.1*
Surface area (m <sup>2</sup> ) placenta	145.3±36.0	237.5±157.9	138.2±97.3	81.3±60.8
Barrier thickness (µm)	14.81±0.95	8.15±2.74*	13.00±1.57	5.78±1.64*
Adjusted barrier thickness	14.81±0.95	19.48±3.29	13.00±1.57	16.18±3.20
Fetal weight (kg)	2.4±0.2	2.9±0.6	4.5±0.2	5.1±0.6

\*denotes significant difference  $p \leq 0.05$  between controls and clones within gestational age group

In clones <135d there were fewer placentomes but these were heavier than in controls. After 135d placentome numbers were also reduced in clones but there was no difference in the mean weight of individual placentomes between the clones and control animals. A large volume of 'shed trophoblast' accompanied by fetal villous haemorrhage was observed in placentomes from cloned animals in both GA groups that was not evident in controls. As a result, there was a decrease of intact trophoblast and the barrier thickness was therefore reduced in clone pregnancies (45% ≤ 134d GA & 56% ≥ 135d GA). The arithmetic mean barrier thickness of trophoblast is a measure of the distance through which substrates are exchanged within the placentome. The thinner the barrier the greater the opportunity for exchange. After adjustment for the volume of shed trophoblast, there was no difference, however, in the barrier thickness of placentomes from clones compared to controls. The surface area of trophoblast for exchange was increased in clones (430% ≤ 134d GA & 98% ≥ 135d GA) and this was a consequence of the increase in placentome weight as the surface density (surface area/g placentome; a measure of how convoluted the exchange surface is) was consistent between the groups.

**Conclusion:** The generation of pregnancies by somatic cell nuclear transfer results in an alteration in the structural development of the placenta. Trophoblast death, shedding and fetal villous haemorrhage may be associated with the high rate of fetal demise associated with this reproductive technology.

## A7

### Differential effects of early pregnancy treatment with natural and synthetic glucocorticoids in sheep.

E.M. Wintour<sup>1</sup>, M.Dodic<sup>1</sup> and KM Moritz<sup>2</sup>.

<sup>1</sup>Depts of Physiology and <sup>2</sup>Anatomy and Cell Biology, Monash University, Clayton Vic 3800

Over the past 10 years we have been studying the effects of treating sheep, early in pregnancy (26-28d; term =145-150d) with the synthetic glucocorticoid, dexamethasone, or the natural steroid, cortisol. Certain outcomes were the same with both treatments, but others varied considerably. These are summarized, and some ideas suggested for the differential effects of the two treatments.

#### *Blood Pressure*

In both treatments the offspring, both male and female, developed high blood pressure from at least 4 months after birth. However, in the case of the synthetic steroid (Dex), this was due, in both sexes, to an increase in cardiac output, largely due to an increase in stroke volume. With cortisol treatment, however, the increased blood pressure was due to an increase in peripheral resistance.

#### *Expression of components of the renin-angiotensin system in the brain.*

In the Dex-treated offspring there was an increased expression of the angiotensinogen gene in the hypothalamus, and of the AT1 receptor in the medulla oblongata. When graded doses of Ang II were infused, via a cerebral ventricle (ICV), at 2-3 years of age, there was a larger pressor response in the Dex than in the control animals, suggesting these gene changes had functional consequences. In contrast, there was no difference in the expression of these genes in the Cortisol-offspring, and no change in the pressor responsiveness to ICV infused Ang II.

#### *Kidney development and gene expression.*

In the offspring of both types of glucocorticoid treatment the kidneys had a reduction in nephron number of ~30%; the expression of the angiotensin receptors was down-regulated in mid-gestation, and up-regulated after nephrogenesis was completed. Other gene changes were qualitatively the same in both treatments.

#### *Hippocampal expression of glucocorticoid (GR) and mineralocorticoid (MR) receptors and the Hippocampal-Hypothalamic-Pituitary Adrenal Axis (HHPA).*

In the Dex-treated offspring there were no changes in the expression of GR and MR in the hippocampus, no difference in activation of the HHPA with the stress of haemorrhage, and no difference in maximal cortisol response to a 5-day IV ACTH infusion. The cortisol-treated offspring have marked increases in GR and MR in the hippocampus, but no testing of the HHPA axis has yet been carried out. One might hypothesize that some decreased activation would be seen with stress imposed on these adults.

#### *Comments*

Synthetic and natural glucocorticoids have differing effects on MR and GR, and differentially activate other receptors, such as the PXR. The above intriguing data suggest more investigations are warranted in these 2 models of 'programmed hypertension'.

# A8

## PRENATAL GLUCOCORTICOIDS AND THE KIDNEY – AN UPDATE.

**Karen Moritz**, D Bartal, A Jefferies, M Dodic, WM Boon, J Bertram, M Wintour and R DeMatteo. Depts of Anatomy & Cell Biol, & Physiology, Monash University, VIC 3800.

**Background:** We have shown that treatment of the pregnant ewe with either dexamethasone (DEX, synthetic glucocorticoid) or cortisol (CORT, natural glucocorticoid) between days 26-28 of pregnancy results in offspring with hypertension. This is associated with multiple gene changes in the late gestation kidney including upregulation of the angiotensin receptors as well as the mineralocorticoid and glucocorticoid receptors. The DEX treatment also causes a significant reduction in glomerular number in adult animals at 7 years of age.

**Aim:** To determine gene expression levels of factors important in normal kidney development at different stages after maternal glucocorticoid treatment and assess if prenatal cortisol causes a nephron deficit. In addition, we examined basal renal function in adult animals exposed to prenatal DEX or CORT.

**Methods:** Ewes were infused intravenously with DEX (0.48mg/h), CORT (5mg/h) or saline (0.19ml/h) for 48 hours (day 26-28 of pregnancy) and were killed at 47, 75 or 140 days of gestation or allowed to lamb. Gene expression was determined using real-time PCR at all fetal ages whilst glomerular number determined using unbiased stereology at 140 days. Basal GFR and renal blood flow were examined over 6 hours in animals at 5 years of age.

**Results:** Treatment with DEX and CORT had no effect on body or kidney weight however both caused a significant decrease in glomerular number (table 1), \*\*P<0.01, \*\*\*P<0.001.

	Saline (n=5)	DEX (n=5)	CORT (n=6)
Body weight (kg)	4.0±0.3	4.0±0.2	3.8±0.2
Right kidney weight (g)	10.1±1.0	10.9±0.7	9.5±1.1
Glomerular number	440082±19352	341806± 9878**	289282±28084***
Individual glom vol (mm <sup>3</sup> x10 <sup>-3</sup> )	0.64±0.03	0.74±0.08	0.89±0.08*

Gene expression for components of the renin-angiotensin system [AT1 receptor, AT2 receptor, renin, angiotensinogen (Aogen)], epithelial sodium channels (ENaC's), Na/K ATPase's, WT-1 and TGF-β were all altered by prenatal glucocorticoid treatment. In general, changes with DEX and CORT were qualitatively similar, however changes in gene expression were age dependent (see table 2). Arrows indicate changes in DEX/CORT groups compared to saline group (n=8-10 in each group).

Age/ Gene	AT1 rec	AT2 rec	Renin	Aogen	ENa C (β)	ENa C (γ)	Na/K (α) ATPase	Na/K(γ) ATPase	Wt-1	TGF- β
47	↔	↔	↑	↔	ND	ND	ND	ND	↔	↔
75	↓	↓	↔	↔	↔	↔	↔	↔	↓	↓
140	↑	↑	↑	↑	↑	↑	↑	↑	ND	ND

In adult sheep at 5 years of age basal GFR and RBF were similar in all groups as was urinary excretion of sodium.

**Conclusions:** Prenatal glucocorticoid exposure causes temporal alterations in gene expression in the kidney months after the exposure. These changes may result in altered nephrogenesis and contribute to the low nephron endowment. However, in the adult basal renal function is maintained. Changes in genes important in renal function indicate that subtle differences may occur when animals exposed to prenatal glucocorticoids are exposed to a challenge such as a high salt diet. Experiments are underway to test this hypothesis.

# A9

## FETAL PROGRAMMING OF A NEPHRON DEFICIT IN THE MOUSE BY MATERNAL PROTEIN RESTRICTION

**Chantal C Hoppe**<sup>1</sup>, Karen M Moritz<sup>1</sup>, Roger G Evans<sup>2</sup> and John F Bertram<sup>1</sup>. Departments of <sup>1</sup>Anatomy & Cell Biology and <sup>2</sup>Physiology, Monash University, Melbourne, Victoria 3800, Australia.

The kidney is particularly susceptible to an adverse *in utero* environment. In several species, a maternal low protein diet results in offspring with a permanent nephron deficit. Elevated blood pressure and aberrant renal function have also been reported. Surprisingly, few studies have examined renal developmental programming in the mouse. We examined the effects of maternal protein restriction on organ weights and nephron number in the mouse.

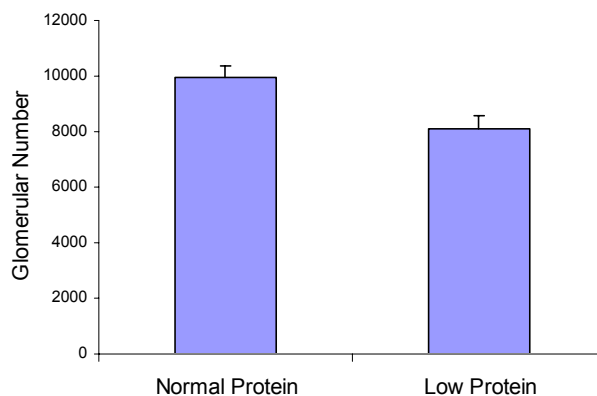
C57/Bl6/129sv mice were fed either a normal (20% w/w) or low (8% w/w) protein diet during gestation and postnatal life. At postnatal day 30, mice were killed and body and organ weights determined. Male left kidneys were embedded in glycolmethacrylate for glomerular number estimation using the disector/fractionator technique.

Kidney, liver, spleen and heart weights in both males and females fed a low protein diet were reduced. Brain sparing was evident in both males and females, but was more marked in females. A 19% nephron deficit was observed in male mice fed a low protein diet *in utero* and postnatal life ( $p < 0.03$ ).

Male	Absolute Values (g)		Normalised Values (mg/g body weight)	
	Normal Protein (n=14)	Low Protein (n=9)	Normal Protein	Low Protein
Body	19.8±0.2	15.9±0.7*	-	-
Right Kidney	0.131±0.003	0.099±0.006**	6.631±0.110	6.199±0.129*
Left Kidney	0.129±0.003	0.092±0.005***	6.512±0.091	5.786±0.187*
Liver	1.191±0.038	0.823±0.039***	60.073±1.556	52.063±1.774*
Spleen	0.106±0.004	0.073±0.004****	5.343±0.201	4.709±0.381
Heart	0.107±0.004	0.090±0.003*	5.373±0.166	5.737±0.228
Brain	0.440±0.003	0.402±0.008*	22.281±0.221	24.863±1.007

Female	Absolute Values (g)		Normalised Values (mg/g body weight)	
	Normal Protein (n=7)	Low Protein (n=10)	Normal Protein	Low Protein
Body	17.3±0.2	12.9±0.3***	-	-
Right Kidney	0.106±0.004	0.073±0.002***	5.990±0.139	5.664±0.069
Left Kidney	0.098±0.003	0.069±0.002***	5.737±0.089	5.365±0.130*
Liver	0.933±0.033	0.813±0.019*	52.827±1.824	63.004±1.371**
Spleen	0.079±0.003	0.062±0.002*	4.480±0.163	4.777±0.207
Heart	0.089±0.002	0.077±0.002*	5.147±0.173	6.014±0.253*
Brain	0.420±0.009	0.407±0.005	24.618±0.471	31.419±0.458***

\* $P < 0.05$ , \*\* $P < 0.001$ , \*\*\* $P < 0.0001$ ; values are mean  $\pm$  SEM



These results show that kidney weight and nephron endowment are programmed in the mouse following a maternal low protein diet and a low protein postnatal diet. We are currently assessing arterial pressure, renal function and global gene expression in this model, with a specific focus on the consequences of “catch-up” growth.

# A10

## Renal Function and Prenatal Programming of Hypertension in the Spiny Mouse

Hayley Dickinson<sup>\*^</sup>, Marelyn Wintour<sup>\*</sup>, David Walker<sup>\*</sup>, Karen Moritz<sup>^</sup>

Departments of <sup>\*</sup>Physiology and <sup>^</sup>Anatomy and Cell Biology, Monash University, Clayton, Victoria, Australia. [Hayley.Dickinson@med.monash.edu.au](mailto:Hayley.Dickinson@med.monash.edu.au)

**Background:** In sheep & rat brief (2 days) exposure of the fetus to the synthetic glucocorticoid, dexamethasone (dex), early in gestation when the kidney is at a pre-glomerular stage of development, results in a decreased total nephron number and hypertension in the adult offspring. It is proposed to develop a third animal model in the spiny mouse (*Acomys Cahirinus*) to test the general application of the findings in sheep and rats. Spiny mice are especially suited to perinatal research as they have a relatively long gestation (39-40 days), few offspring (1-3) and in many aspects of development (eg. placenta, brain, lung and kidney) are more similar to the human than other rodents.

**Aims:** 1. To investigate the effect of treating pregnant spiny mice at a 'preglomerular' stage in kidney development with Dexamethasone for 60hrs on kidney structure, function and blood pressure in young adult offspring

2. To examine the renal structure and function of the spiny mice, a desert adapted species

3. To describe placental development throughout gestation in the spiny mouse

**Methods:** Kidneys were collected from adult spiny mice and glomerular number, volume and cortex to medulla ratios determined using unbiased stereology. Dex or saline is administered to pregnant spiny mice by implanting an Alzet mini-pump subcutaneously on day 20 of gestation. Blood pressure is measured in the offspring at 19 weeks of age via a carotid artery catheter connected to a swivel and pressure transducer, for 1 week. Kidneys are collected and processed for stereological examination to determine volume and nephron number.

Young adult spiny mice were placed in metabolic cages for 24hrs and urine collected for further analysis. Placentas were collected from spiny mice at day 14 through to day 39 of gestation and processed for histological and immunohistochemical examination.

### **Results:**

<b>Stereological Analysis</b>	<b>Spiny Mice (n=8)</b>	<b>C57/BL Mice (n=4)</b>
Volume (mm <sup>3</sup> )	0.106±0.007 **	0.160±0.011
Cortex to Medulla Ratio	1.276±0.065:1 **	1.671±0.053:1
Glomerular Number	7245±280 ***	11421±536
Glomerular Volume (mm <sup>3</sup> ×10 <sup>-4</sup> )	3.634±0.184 ***	2.298±0.168
Total Glom. Volume (mm <sup>3</sup> ×10 <sup>-3</sup> )	2.620±0.147	2.619±0.194
Corpuscle Volume (mm <sup>3</sup> ×10 <sup>-4</sup> )	3.755±0.216 **	2.512±0.147
Total Corpuscle Volume (mm <sup>3</sup> ×10 <sup>-3</sup> )	2.708±0.174	2.859±0.162

Preliminary examination of spiny mouse placentas suggests that it develops in a similar fashion to that of the laboratory mouse, although the time scale is significantly lengthened.

**Work in Progress:** Blood pressure data is currently being analysed in male and female dex and saline animals. Stereology is being used to determine total nephron number in treated and control offspring. Urine collected from spiny mice placed in metabolic cages is being analysed for urinary electrolytes and osmolality. Placentas are undergoing further examination to determine the changes in area of labyrinth and junctional zone throughout gestation.

**Conclusion and Future Studies:** Spiny mice are a desert adapted species and preliminary examination have shown a kidney structure significantly different to that of the c57/black mouse. Future studies are aimed at determining renal function (GFR, Effective renal plasma flow and urine flow) in 19 week old spiny mice with kidneys being perfused fixed for vascular casts and electron microscopy.

# A11

## Programming of the renal sympathetic nerves in offspring of hypertensive mothers

KM Denton<sup>1</sup>, RL Flower<sup>1</sup>, GW Lambert<sup>2</sup>, EM Wintour<sup>1</sup>, D Maduwegedera<sup>1</sup>

<sup>1</sup>Department of Physiology, Monash University, <sup>2</sup>Baker Heart Institute, Victoria, Australia

Chronic hypertension complicates ~ 5% of pregnancies and is associated with increased rates of adverse fetal outcomes, including low and high birth weight. Compelling evidence supports the hypothesis that blood pressure is programmed *in utero*. Thus the question of whether hypertension during pregnancy predisposes the offspring to an increased risk of cardiovascular disease in later life is of great consequence. Indeed, we have shown that adult rabbit offspring of mothers with chronic hypertension have increased blood pressure<sup>1</sup>. Strong evidence indicates that renal sympathetic nerves and the renin-angiotensin system are implicated in the pathogenesis of hypertension<sup>2</sup>. Thus we have examined renal noradrenaline content (estimation of renal sympathetic innervation) in pre-hypertensive rabbit offspring of two kidney, one wrap hypertensive (2K-1W; n=5) and sham operated (n=5) mothers at birth and at 5 weeks of age. Conscious mean arterial pressure (MAP) and plasma renin activity (PRA) were measured at 5 weeks.

Litter size and gestational age were not significantly different between groups. Birth weight of 2K-1W (62±5 g; P<0.05) was significantly greater than sham offspring (43±3 g). Brain (10%; P<0.05), liver (30%; P<0.05) and kidney (30%; P<0.05), but not heart weights were increased in the 2K-1W compared to sham offspring. At 5 weeks of age, there was no difference in body weight, organ weight or MAP but PRA was suppressed (3.63±0.5 vs. 5.01±0.5 ngAI/ml plasma/h, P<0.05) in offspring of 2K-1W compared to sham mothers.

Kidney noradrenaline content was significantly higher in female as compared to male normotensive offspring. Kidney noradrenaline content was reduced by 40% in the offspring of the hypertensive mothers (332±50 ng/g; P<0.05) as compared to sham (524±34 ng/g) at birth, but was not significantly different at 5 weeks of age. Stratification of data into the sexes demonstrated the decrease in NA content was seen primarily in the female offspring of hypertensive mothers.

Renin and NA levels surge at birth; however these data indicate a suppression of renal sympathetic and renin-angiotensin systems in young offspring of hypertensive mothers, possibly reflecting an accelerated maturation, which may play a role in the programming of hypertension in these offspring.

Denton et al, Hypertension 41:634-9, 2003

Esler et al, J Cardiovasc Pharmacol 26:S24-8, 1995

# A12

## EFFECTS OF FETAL LUNG HYPOPLASIA (LH) ON THE STRUCTURE OF PULMONARY VASCULATURE IN SHEEP

Keiji Suzuki, Stuart B Hooper, Richard Harding. Department of Physiology, Monash University, Melbourne, Victoria, Australia. E-mail: dks@med.monash.edu.au

**Background and Objectives:** We previously reported that, in our sheep model of fetal lung hypoplasia (LH), the change in pulmonary vascular resistance (PVR, ~140% increase) was greater than the changes in lung size and compliance (~30% decrease). The aim of this study was to investigate morphological changes in the pulmonary vasculature in LH which could provide an explanation for this great change in PVR.

**Subjects and Methods:** LH was induced in 6 ovine fetuses by the creation of a tracheo-amniotic shunt as well as amniotic fluid drainage starting at  $105.5 \pm 6.1$  (mean  $\pm$  SD) days of gestation (term ~147 days). At  $140.0 \pm 1.3$  days, the fetal lambs were delivered and ventilated for two hours, during which systemic and pulmonary artery pressures, left pulmonary artery blood flow and measures of respiratory function were recorded. At autopsy, the right lung was fixed via the trachea at 20 cmH<sub>2</sub>O. Paraffin-embedded sections of 4- $\mu$ m thickness were stained using Masson's trichrome and Gomori's aldehyde fuchsin methods. The images were taken at 100x magnification and analysed using a computer image analysis program. All arteries and veins with diameter  $\geq 25\mu$ m were examined. Volume density of arteries and veins in lung tissue (Vat, Vvt; total area occupied by arteries or veins relative to the area occupied by tissue alone) were analysed. For arteries with diameter of  $<75\mu$ m, the relative medial wall thickness of arteries (%MWT; medial wall thickness/diameter of the artery x100) was also calculated. Volume density of lumen of arteries in the lung tissue (Vat-lm) was estimated from Vat and %MWT values. Mean terminal bronchiole density (MTBD; mean number of terminal bronchioles per microscopic field, an indicator of airway development) was also analysed for assessment of airspace development.

**Results:** In LH lambs compared to controls, volume density of arteries was ~30% lower and volume density of veins was ~45% lower. For the arteries with diameter  $<75\mu$ m, %MWT was 16% higher and estimated volume density of lumen of arteries was ~45% lower. TBD was 32% higher.

	LH (n=6)	Control (n=6)
Vat (x10 <sup>-3</sup> )	10.5+/-2.2*	15.1+/-2.6
Vat (<75 $\mu$ m) (x10 <sup>-3</sup> )	4.17 +/- 0.86	6.03 +/- 2.27
Vvt (x10 <sup>-3</sup> )	14.1+/-6.9	26.5+/-12.7
%MWT (<75 $\mu$ m) (%)	22.9+/-1.2**	19.8+/-1.0
Vat-lm (<75 $\mu$ m) (x10 <sup>-3</sup> )	1.23 +/- 0.30*	2.20 +/- 0.82
MTBD (/ 6.245mm <sup>2</sup> )	2.12 +/- 0.35*	1.60 +/- 0.44

\*\*p <0.01, \*p <0.05

**Conclusions:** In this LH model, we found a decrease in the volume density of pulmonary arteries and veins and an increase in arterial medial wall thickness (muscularization) which paralleled the increase in PVR.

## A13

### CHANGES IN FETAL DUCTUS ARTERIOSUS FLOW IN UTEROPLACENTAL INSUFFICIENCY.

**Nayana Parange**, Chris Wilkinson, Gustaaf Dekker

Department of Obstetrics and Gynaecology, University of Adelaide, Women's & Children's Hospital, 72 King William Rd, North Adelaide SA 5006.

Email: [nayana.parange@adelaide.edu.au](mailto:nayana.parange@adelaide.edu.au)

Uteroplacental insufficiency (UPI) has long been recognized as a major factor influencing perinatal outcome. Management of a compromised fetus is a challenge to the treating obstetrician as the risks of prematurity have to be balanced against the risk of prolonged fetal exposure to chronic hypoxaemia and acidaemia, leading to adverse perinatal outcome such as severe fetal growth restriction and fetal or neonatal death.

Doppler ultrasound has been extensively employed in fetal surveillance and evidence suggests that longitudinal ultrasound and doppler monitoring of the growth-restricted fetus improves long-term morbidity and mortality by optimal timing of delivery.

It is now understood that the fetus adapts to a low supply of oxygen and nutrients by a preferential redistribution of flow. However, the earliest predictor of fetal hypoxia is still not known. The aim of this study is to test the hypothesis that the fetal ductus arteriosus flow velocity waveforms are the earliest predictor of fetal hypoxia.

A prospective, observational, longitudinal cohort study has been designed to determine the relationship between doppler of fetal and maternal circulation and prediction of adverse perinatal outcome. The study aims to recruit 100 high-risk women and 100 controls every year, for a period of three years. Doppler waveforms of fetal middle cerebral artery, umbilical artery and fetal shunts—ductus arteriosus, ductus venosus and foramen ovale and maternal uterine artery flows will be serially evaluated. These flows will then be correlated with perinatal outcome, perinatal morbidity and mortality.

We hope the results of this study will aid our understanding of fetal adaptation to uteroplacental insufficiency in utero and establish which of the doppler parameters is the best predictor of early fetal compromise. It will enable clinicians to identify the fetuses at risk of compromise very early and decide the best parameter for monitoring the high risk fetus.

## A14

### ASSESSMENT OF BLOOD VOLUME PARAMETERS IN PERICONCEPTIONALLY UNDERNOURISHED PREGNANT EWES

**Rumball CWH**, Van Zijl P, Bloomfield FH, Harding JE  
Liggins Institute, Faculty of Medical and Health Sciences, University of Auckland,  
Auckland, New Zealand. [c.rumball@auckland.ac.nz](mailto:c.rumball@auckland.ac.nz)

**Aims:** To assess whether maternal periconceptional undernutrition of varying timing and duration alters the normal physiological changes in blood volume (BV) that occur during pregnancy, and to develop a method for measuring blood volume that is accurate and can be performed in a variety of locations.

**Methods:** Romney ewes were allocated to one of four nutritional groups: control, undernourished (UN) from 60 days before (-60d) until 30 days after (+30d) mating, UN from -60d to mating, or UN from mating to +30d. All ewes were fed concentrate feed and weighed twice weekly. The feed of undernourished ewes was adjusted individually to reduce body weight by 10-15%. At 65 and 120 days gestation, blood volume parameters were estimated in singleton-bearing pregnant ewes using the method described below.

Fluorescein-labelled dextran (FD250S, Sigma-Aldrich, St Louis, USA), dissolved in 10 ml saline at 4 mg/ml, was injected as an intravenous bolus and blood samples taken every ten minutes for one hour. Plasma FD250S concentrations were measured in microplates by fluorimetry, with a standard curve on each plate. Using a semilog graph, concentration at time 0 was determined, and plasma volume (PV) obtained by dividing the amount given by this concentration. BV was then calculated using the whole body haematocrit. In four sheep at 120 days gestation BV results were compared between different and repeated doses of dextran (40 mg and 175 mg FD250S), and <sup>51</sup>Cr-labelled red cells.

**Results:** In the four sheep where we obtained three measurements using FD250S on the same day, the coefficient of variation for BV was  $2.4 \pm 0.3\%$ . Different doses gave similar results. BV derived from the radiolabelled red cell method tended to be higher than those derived from this dextran method (mean difference  $180 \pm 145$  ml) but this 3.7% variation is neither statistically nor clinically significant.

There were no significant differences between nutritional groups in blood volume, plasma volume, or red cell volume (RCV). Across all groups there was a mean increase in PV from day 65 to 120 of  $7.4 \pm 1.7$  ml/kg ( $p=0.001$ ) and decrease in RCV of  $2.5 \pm 1.1$  ml/kg ( $p=0.03$ ).

#### **Conclusions:**

Fluorescein-labelled dextran can be used to estimate blood volume parameters in pregnant sheep.

Periconceptional undernutrition does not appear to have large effects on parameters of intravascular volume in pregnant sheep. PV increases and RCV decreases from day 65 to 120 in pregnant sheep.

# A15

## ENDOTOXIN IMPAIRS ON CEREBRAL CIRCULATION DURING REM SLEEP IN NEWBORN LAMBS

**Feng, YS**<sup>1,2</sup>, Cassaglia P<sup>1</sup>, Zivkovic, S<sup>1</sup>, Yu, VYH<sup>1,2</sup>, Walker, AM<sup>1</sup>

<sup>1</sup>Ritchie Centre for Baby Health Research, Monash University, Monash Institute of Reproduction and Development, Monash University, Clayton, Melbourne, Victoria, 3168, Australia

<sup>2</sup>Newborn Services, Monash Medical Centre, 246 Clayton Rd Clayton, VIC 3168, Australia.

**Email:** [ying.feng@med.monash.edu.au](mailto:ying.feng@med.monash.edu.au)

**Introduction:** Little is known of the regulation of cerebral blood flow (CBF) during sleep, though human beings spend one-third of their lives sleeping, and despite the significant clinical problems posed by sleep disorders, such as sudden infant death syndrome (SIDS). It has been demonstrated in pathology that bacterial toxins play a potential role in SIDS causation, but the pathophysiology of the brain during sleep remains unknown. In this study we assessed the cerebral circulation during the highly variable rapid-eye-movement (REM) sleep phase following a lipopolysaccharide (LPS) induced inflammatory insult.

**Aim:** To assess cerebral circulation during arterial blood pressure (ABP) surges in REM sleep before and after exposure to LPS.

**Methods:** Lambs (n = 6) of age 1-4 weeks old were instrumented under general anaesthesia (halothane 1.5%, oxygen 50%, balance N<sub>2</sub>O) for recording sleep-wake state, CBF (Transonic™ flow probe on the superior sagittal sinus), carotid arterial pressure (Pca) or femoral arterial pressure (Pfa), and intra-cranial pressure (Pic). A jugular venous (JV) catheter was implanted for drug administration. Sleep studies were performed before and after LPS infusions (2µg/kg over 30 min) on three consecutive days. Circulatory changes during ABP surges (defined as Pca or Pfa increases exceeding 15%) in REM were analysed. Cerebral vascular resistance (CVR) was calculated as (Pca – Pic)/CBF, or (Pfa-Pic)/CBF.

**Results:** A total of 34 ABP surges were analysed, comprising 21 pre-LPS, and 13 post-LPS. At the peak of the surge  $\Delta$ ABP (mean  $\pm$  SEM) was  $26 \pm 3\%$ ,  $\Delta$ CBF was  $48 \pm 3\%$  ( $P < 0.001$ ) and  $\Delta$ CVR was  $-14 \pm 2\%$  ( $P < 0.001$ ). Following LPS treatments,  $\Delta$ ABP was similar ( $26 \pm 3\%$ ), but both  $\Delta$ CBF ( $31 \pm 4\%$ ) and  $\Delta$ CVR ( $-3 \pm 3\%$ ) were significantly reduced ( $P=0.01$  and  $P<0.05$  respectively, One Way Repeated Measures ANOVA).

**Conclusion:** LPS impairs the cerebral vasodilatation and blood flow increment during the characteristic blood pressure surges of REM sleep in newborn lambs. This is new information and helps in further understanding endotoxin-mediated cerebral dysfunction. Further study is required to explore the basis of the LPS-induced cerebral haemodynamic dysfunction, including the possibility of endothelial injury.

**Key words:**

cerebral circulation, REM sleep, lipopolysaccharide

## A16

### Effects of Intrauterine Growth Restriction on Cardiomyocyte Maturation and Function of Small Coronary Arteries.

**KJ Bubb**, ML Cock, \*MJ Black, HC Parkington, M Dodic, R Harding and M Tare.

Fetal and Neonatal Research Group, Department of Physiology and \*Department of Anatomy and Cell Biology, Monash University, Clayton, Victoria, Australia.

**Introduction:** Intrauterine growth restriction (IUGR) affects up to 10% of pregnancies and has been linked to an increased risk of cardiovascular disease later in life. IUGR can lead to underdevelopment of the heart and if severe, can impair cardiac function in the newborn. To date, however, there is little information on the effects of IUGR on the growth and maturation of fetal cardiomyocytes prior to birth, or on the repercussions of IUGR on the function of small coronary arteries. Our aims were to investigate whether IUGR impairs the maturation of cardiomyocytes and alters the function of small coronary arteries.

**Methods:** IUGR was induced in catheterised fetal sheep by umbilico-placental embolisation (UPE) with microspheres from 110 days to 130 days of gestation (term~147d); control sheep received saline only. At post mortem, cardiomyocytes were isolated, fixed and nucleation determined. Coronary vascular function was tested by mounting a small branch of the anterior interventricular branch of the left descending coronary artery on a wire myograph to study the endothelial and smooth muscle function. The passive wall mechanics of the coronary artery were examined in vessels mounted on a pressure myograph.

**Results:** Following UPE, fetuses were hypoxemic and hypoglycemic and at 130 days, fetal body weight was lower in the IUGR group ( $2.6 \pm 0.2$ kg) compared with the control group ( $3.5 \pm 0.3$ kg;  $p=0.035$ ). There was no difference in heart weight following IUGR, once adjusted for body weight. The IUGR group had a decreased proportion of binucleated cardiomyocytes in the left ventricle: control ( $n=6$ )  $33 \pm 6\%$  vs IUGR ( $n=7$ )  $21 \pm 4\%$  ( $p=0.02$ ). However, there was no difference between the control and IUGR groups in the cross-sectional area of mononucleated (control  $221 \pm 12 \mu\text{m}^2$  vs IUGR  $215 \pm 12 \mu\text{m}^2$ ;  $p=0.72$ ) or binucleated (control  $318 \pm 21 \mu\text{m}^2$  vs IUGR  $305 \pm 16 \mu\text{m}^2$ ;  $p=0.63$ ) cardiomyocytes. In the coronary arteries, the responses to the vasoconstrictors angiotensin II ( $p<0.01$ ) and U46619 ( $p=0.03$ ) were enhanced in the IUGR group compared with controls. The total endothelium-dependent relaxation in response to bradykinin was not different between the groups. However, the component of relaxation attributable to endothelium-dependent hyperpolarizing factor (EDHF) was larger in IUGR fetuses ( $p=0.03$ ). The coronary arteries were more compliant in IUGR fetuses compared with controls as indicated by a rightward shift in the stress-strain relationship ( $p=0.02$ ).

**Conclusion:** The decreased proportions of binucleated cardiomyocytes suggests that the rate of maturation following IUGR is stunted or delayed. The more compliant small coronary arteries of IUGR fetuses may also be reflective of decreased maturation of the arteries. Increased coronary artery contraction to angiotensin II and U46619 suggest upregulation of pro-constrictor pathways. The increased EDHF component of the relaxation in the IUGR group suggests an adaptation to compensate for reduced bioavailability of other endothelial vasodilators *in vivo*. This study has demonstrated that IUGR has profound effects on the myocardium and coronary vasculature in the fetus and may contribute to increased vulnerability to cardiac and coronary disease in postnatal life.

# A17

## Genomic And Proteomic Applications In Kidney Development.

**Wee-Ming Boon**<sup>1</sup>, Karen Moritz<sup>2</sup>, Ian Smith<sup>3</sup>, Marelyn Wintour<sup>1</sup>.

Department of Physiology<sup>1</sup>, Anatomy and Cell Biology<sup>2</sup>, Biochemistry and Molecular Biology<sup>3</sup>, Monash University, Clayton, VIC3800, Australia.

The fields of proteomics and genomics form some of the most efficient large-scale exploratory tools available to fetal/neonatal physiologists and the biological sciences community as a whole. We are currently investigating the mechanisms of nephrogenesis using these techniques. The two opposing but complementary models of kidney development used are as follows.

**Model 1:** 30-40% overall reduction in nephron endowment and subsequent development of hypertension in adult sheep resulting from exposure to glucocorticoids (days 26-28 of gestation).

**Model 2:** Compensatory increase (30-40%) in nephrogenesis occurring in the one remaining kidney of fetal sheep that had undergone unilateral nephrectomy at day 100 of gestation.

A range of techniques will be applied to these two models including 2-dimensional electrophoresis, microarray analysis, surface-enhanced laser desorption ionization-time of flight (SELDI-TOF) analysis to produce a global 'scan' of the genes and proteins expressed as a result of two separate intrauterine perturbations resulting in two opposing phenotypes (increased nephrogenesis and decreased nephrogenesis). By using two opposing models, we will be able to use the datasets as 'filters' or confirmatory tools to allow a more targeted approach in our investigations. We aim to establish the mechanisms involved in the reduction of nephron number which is associated with hypertension and also the compensatory increase in nephron number as a result of unilateral nephrectomy.

We will provide a (work in progress) review of some of the proteomic and genomic techniques in relation to the two animal models that we are investigating, and discuss their utility in the face of different requirements and experimental constraints.

## PERINATAL ROFECOXIB EXPOSURE IMPAIRS NORMAL RENAL DEVELOPMENT OF OFFSPRING IN ADULTHOOD

Leewen Rattanatray, Jeff Schwartz and David Olson

Discipline of Physiology, School of Molecular and Biomedical Science, Adelaide University  
Perinatal Research Centre, University of Alberta, Edmonton, Canada

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Prostaglandins (PGs) are synthesised by the action of enzymes called cyclooxygenases (COXs) and they play important roles in the regulation of many organ systems postnatally. The roles of PGs in development, aside from maintaining the patency of the ductus arteriosus, are largely unknown. The COX-2 isoform is interesting since its expression is inducible and is present in various organs only at specific times during development. Studies involving COX-2 null mice and with chronic pharmacological COX-2 inhibition during development are consistent with COX-2 playing an important role in normal kidney development. However, neither the precise temporal requirement for COX-2 nor the long-term consequences of COX-2 inhibition during development have been previously assessed.

The aim of this study was to determine the effects of COX-2 inhibition at various stages of development, by feeding a selective COX-2 inhibitor, rofecoxib, to maternal rats during specific stages of gestation and lactation and then measuring anatomical and physiological effects in offspring from birth to adulthood. Pregnant Sprague Dawley rats (term = 22d) were randomly assigned (4-5 per group) to a control group (A), which was fed vehicle daily by orogastric tube from day 1 of pregnancy to postnatal day 7 or one of the experimental groups B-E), which received rofecoxib (10mg/kg per day) instead of vehicle during either week 1 (group B), 2 (C) or 3 (D) of gestation or postnatal week one (E). The effects of COX-2 inhibition on the size and morphology of the kidneys and heart were measured at birth, on postnatal days 7 and 21 and in adulthood (4-5 months). Renal function in the adult offspring was assessed using  $^3\text{H}$  inulin after surgical implantation of vascular and bladder catheters. Glomerular (nephron) densities and maturities were obtained by histology. Results from all offspring in a litter were averaged to yield  $N=1$ ; analyses were performed on the means of litters. I hypothesised that COX-2 inhibition will alter normal renal physiology with permanent consequences, depending on when the inhibition occurs.

Maternal rofecoxib ingestion did not alter birth measurements or litter size. The kidney weight:body weight ratio of rofecoxib-exposed offspring was indistinguishable from the control at all ages assessed. However offspring exposed to rofecoxib during the last week of gestation (Group D) were born with significantly larger hearts ( $0.80\pm 0.10$ ) than controls ( $0.64\pm 0.05$ ). This trend, however, did not persist to postnatal day 7 or later. There were no differences among groups in terms of nephron density at birth or day 7, but at day 21 nephron density was decreased by 27% in offspring that had been exposed to rofecoxib mid-gestation (Group C). Overall, adult offspring GFR was not significantly altered by maternal rofecoxib administration, although there was a trend in mid-gestation exposure (group C) to lower GFR by a third. Interestingly, group C kidneys are of largely normal appearance, whereas by adulthood there was an increase of gross abnormal kidney morphology in the adult offspring exposed to rofecoxib late gestation (Group D) ( $p<0.069$ ).

This study shows that inhibition of COX-2 during mid to late gestation alters nephron formation and or loss such that nephron density is relatively lower by three weeks of age, and late gestational COX-2 inhibition causes an increase incidence of abnormal kidney morphology, which becomes evident only during adolescence and adulthood. The trends in this early study suggest that the anatomical changes may contribute to altered renal function, but further experimentation is required.

# A19

## Exploring mechanisms through which glucocorticoids affect the growth and development of the kidney.

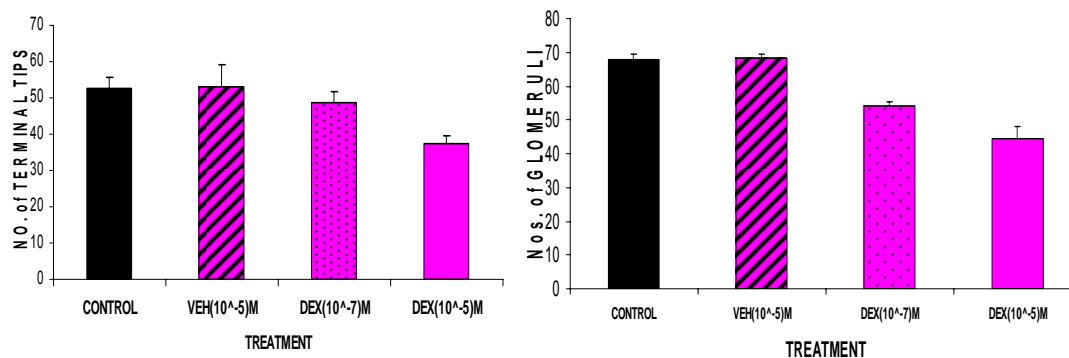
**Reetu Singh**, Karen Moritz, John F Bertram, Luise Cullen-McEwen  
Department of Anatomy and Cell Biology, Monash University, Clayton, 3800.

**Background:** The developmental origins of adult disease hypothesis proposes that fetal adaptations to a suboptimal intrauterine environment programs for adult disease. Prenatal glucocorticoid exposure in both the rat and sheep results in offspring with a reduced nephron endowment and hypertension. However, the mechanism through which prenatal glucocorticoids result in a decrease in nephron number is unknown. The aim of this study was to determine the effects of exogenous glucocorticoids on branching morphogenesis in the developing metanephric kidney in culture, as nephrogenesis only occurs adjacent to branch tips.

**Hypothesis:** Glucocorticoids cause a decrease in nephron endowment by decreasing ureteric branching morphogenesis.

**Methods:** Metanephric kidneys were dissected from embryonic day 14 (E14) Sprague-Dawley rats and cultured in control media or media containing dexamethasone (DEX,  $10^{-7}$ M,  $10^{-5}$ M) or corticosterone ( $10^{-6}$ M,  $10^{-4}$ M) for 2 days. As the glucocorticoids were dissolved in ethanol, kidneys were also cultured in media containing equivalent doses of ethanol (vehicle control). After 2 days the number of branch points and branch tips were determined. Some kidneys remained in culture in control media for a further 3 days after which glomerular number was determined.

**Results:** Culture with DEX inhibited branching morphogenesis at  $10^{-5}$ M ( $P < 0.05$ ) and glomerulogenesis (both  $10^{-7}$  and  $10^{-5}$ M,  $P < 0.001$ , see figure below).



Corticosterone did not significantly affect branch points, terminal tips or glomerular number after 48 hours at  $10^{-6}$ M. At  $10^{-4}$ M the vehicle control caused a significant decrease in values for all parameters. However, culture with corticosterone at  $10^{-4}$ M significantly reduced glomerular number by 35% compared to the vehicle (ethanol) control at  $10^{-4}$ M.

**Conclusions:** DEX inhibits branching morphogenesis and nephrogenesis in the rat kidney in culture. This suggests DEX may act directly to inhibit branching morphogenesis which results in a nephron deficit. Corticosterone may cause a decrease in nephron number but may involve mechanisms other than branching morphogenesis. Interestingly, ethanol (alcohol) may also directly affect branching of the ureteric tree of the metanephric kidney.

## NET FILTRATION PRESSURE AND TUBULOGLOMERULAR FEEDBACK IN FETUSES AND LAMBS

**Karen J. Gibson\***, Russell D. Brown\*<sup>+</sup>, Anita J. Turner\* and A. Erik G. Persson\*<sup>+</sup>,

\* Dept of Physiology & Pharmacology, School of Medical Sciences, University of New South Wales, Sydney, 2052. <sup>+</sup>Dept of Physiology, Uppsala University, Uppsala, Sweden.

**Introduction:** Glomerular filtration rate increases greatly in the first week after birth<sup>1</sup>. To greater understand the mechanisms responsible for this increase, we have measured net filtration pressure and the tubuloglomerular feedback response (TGF) in late gestation fetuses (age 133-143 days) and lambs at 5-17 days after birth.

**Methods:** Micropuncture studies were carried out in anesthetized fetuses (133-143 days) and lambs (5-15 days after birth). For the fetal studies, the fetus was exteriorized in a shallow heated water bath and the cord was kept warm, moist and unstretched. The lambs were studied on a thermostatically controlled heating mat. In both groups the left kidney was exposed, stabilized in a perspex cup, viewed with a dissecting stereomicroscope and the outer layers of the renal capsule were carefully removed over a portion of the kidney cortex. Both fetuses and lambs were infused with normal saline at maintenance levels (5 ml/kg/h) and vecuronium (0.1 mg/kg) was administered as needed. Early proximal tubular segments were identified and punctured with a sharpened glass pipette (outer diameter ~ 5  $\mu$ m) filled with 1M NaCl stained with lissamine green, using a micromanipulator. The pipette was connected to a servo-nulling pressure system so that intratubular pressure could be measured. Pressure was measured initially under free flow conditions ( $P_{ff}$ ). Then the tubule distal to the pipette was blocked with wax, and additional readings were taken until a new stable pressure, the stop-flow pressure ( $P_{SF}$ ) was reached. TGF was studied by measuring  $P_{SF}$  in response to perfusion of the loop of Henle at rates of 0-40 nl/min.

**Results:** Mean arterial pressure was  $49.3 \pm 1.6$  mmHg (s.e.m., n=22) in the fetuses and  $71.3 \pm 3.5$  (n=11,  $P < 0.001$ ) in the lambs. In the fetuses,  $P_{ff}$  was  $6.0 \pm 0.1$  mmHg (n=tubules/animals, 111/22), which was lower than in the lambs ( $9.1 \pm 0.2$ , n=50/11,  $P < 0.001$ ). Fetal and lamb  $P_{SF}$  were  $26.5 \pm 0.9$  mmHg (n=20/8) and  $33.8 \pm 0.6$  (n=31/8,  $P < 0.001$ ) respectively. Thus, net filtration pressure ( $P_{SF} - P_{ff}$ ) was lower in fetuses than lambs ( $20.1 \pm 1.1$ , n=13/8, versus  $24.7 \pm 0.8$  mmHg, n=16/8,  $P < 0.005$ ).

Both fetuses and lambs exhibited TGF. In fetuses the mean maximum change in  $P_{SF}$  ( $\Delta P_{SF}$ ) was  $4.8 \pm 0.5$  mmHg (n=11/8) with a turning point (TP) at  $15.7 \pm 0.6$  nl/min (n=7/4). In lambs these values were  $10.5 \pm 0.5$  mmHg (n=23/8,  $P < 0.001$ ) and 19.4 nl/min respectively (n=11/7,  $P < 0.02$ ).

**Conclusions:** It is concluded that net filtration pressure increases between fetal and neonatal life. This rise would contribute to the fourfold increase in glomerular filtration rate observed at this time. Furthermore, we have shown that TGF is active in fetal life as well as after birth. However in fetal life TGF operates with reduced reactivity (i.e.  $\Delta P_{SF}$  is lower) and greater sensitivity (i.e. TP is lower) than after birth.

<sup>1</sup>Robillard *et al.* (1981). Ontogeny of single nephron glomerular perfusion rate in fetal and newborn lambs. *Pediatric Research*, 15, 1248-1255.

## A21

### **Renal function, arterial pressure and body growth in lambs born to subtotally nephrectomised mothers.**

**O'Connell, AE**, Boyce, AC and Gibson, KJ. Department of Physiology and Pharmacology, School of Medical Sciences, University of New South Wales, Sydney, NSW, Australia, 2052.  
a.oconnell@student.unsw.edu.au

**Background:** Renal disease in pregnancy is associated with an increase in fetal morbidity and mortality. We have developed a model of maternal renal disease in which non pregnant ewes underwent subtotal nephrectomy (STNx). This involves the removal of the right kidney and partial infarction of the left kidney by ligating a branch of the renal artery. We have shown previously that renal function was altered in fetuses whose mothers underwent this procedure<sup>1</sup> (STNxF), with a doubling in urine flow rate and sodium excretion compared to control fetuses. This, together with their lower haematocrit and a suppression of the renin angiotensin system (RAS)<sup>2</sup>, suggests that these fetuses were volume expanded. Furthermore, the kidneys of STNxF were wider, although kidney to body weight ratio were similar between groups.

**Aim:** To determine if the differences in renal function and the volume expansion that occurred *in utero* in offspring of STNx ewes persist after birth.

**Methods:** Ewes underwent subtotal nephrectomy at least 6 weeks prior to mating. Lambs from STNx mothers (STNxL) and intact mothers (ConL) underwent surgery 3-7 days after birth. Catheters were placed in the femoral artery and vein, as well as the bladder. Baseline renal and cardiovascular parameters were measured 3-6 days after surgery at 7-14 days old. After the experimental procedure a post mortem was conducted and body weight, dimensions and organ weights were measured.

**Results:** Studies were carried out in 10 ConL and 9 STNxL. There were no differences in the renal or cardiovascular parameters that were measured. Urine flow rate and the excretion and clearances of sodium, potassium and osmoles were not different between the two groups. Mean arterial pressure and heart rate were similar in both groups (MAP ConL  $76.0 \pm 1.7$  v STNxL  $76.3 \pm 2.0$  mm Hg; HR ConL  $232 \pm 9$  v  $217 \pm 14$  beats/min) and there was also no difference in the haematocrit. Glomerular filtration rate was similar between the two groups whether it was expressed in absolute terms (ConL  $29.5 \pm 3.0$  v STNxL  $31.2 \pm 5.9$  ml/min), or relative to body weight and kidney weight. At post mortem, there was no difference in the weights of the animals and they had similar nose-rump lengths and abdominal girths. Kidney to body weight ratio was not different from controls (ConL  $5.8 \pm 0.3$  v STNxL  $5.7 \pm 0.2$  g/kg body weight); neither were the kidneys dimensions (Length ConL  $4.5 \pm 0.1$  v STNx  $4.8 \pm 0.1$  cm; Width ConL  $2.3 \pm 0.1$  v  $2.4 \pm 0.1$  cm). Other organ to body weight ratios were similar between the two groups.

**Conclusions:** The diuresis, natriuresis and reduced haematocrit seen in late gestation fetuses of STNx ewes was not seen 1-2 weeks after birth. Thus, following removal from exposure to an excess transplacental fluid and solute load, these lambs were able to resolve the volume expansion that had developed in utero. We are currently assessing the capacity of these animals to respond to various challenges of renal and cardiovascular function as well as stimulation of renin release.

1. Gibson *et al.* (2003). APPS Conference, 12OP;
2. Gibson *et al.* (2004). PSANZ 8<sup>th</sup> Annual Conference.

**GENDER SPECIFIC PROGRAMMING OF PEROXISOME-PROLIFERATOR ACTIVATED RECEPTOR- $\gamma$  AND LEPTIN mRNA EXPRESSION IN VISCERAL FAT IN LOW BIRTH WEIGHT LAMBS**

*JA Duffield<sup>1</sup>, T Vuocolo<sup>2</sup>, R Tellam<sup>2</sup>, JR McFarlane<sup>3</sup>, KG Kauter<sup>3</sup>, BS Muhlhausler<sup>1</sup>, IC McMillen<sup>1</sup>*

*<sup>1</sup>Department of Physiology, Adelaide University, SA 5005, Australia, <sup>2</sup>CSIRO Livestock Industries Brisbane, Queensland, Australia, <sup>3</sup>Department of Animal Science, University of New England, NSW, Australia*

**Objective:** Epidemiological studies have shown that a low birth weight coupled with a rapid postnatal growth rate is associated with an increased adiposity in adult life. We have investigated the impact of low birth weight and gender on the expression of genes that regulate the differentiation (PPAR $\gamma$ , RXR $\alpha$ ), insulin sensitivity (adiponectin) and lipid metabolism (leptin, LPL, G3PDH) of perirenal adipocytes in lambs at 21d of life. **Methods:** Lambs were separated into low birth weight (LBW, <4.4 kg, n=9) and average birth weight (ABW, >4.5kg, n=15) groups. An Insulin RIA and competitive ELISA for leptin were used for plasma analyses. The relative quantity of PPAR $\gamma$ , RXR $\alpha$ , leptin, adiponectin, LPL, and G3PDH mRNA in the perirenal fat depot was determined by qRT-PCR, and the mean size of adipocytes was determined using standard image analysis. **Results:** There was no difference between LBW and ABW lambs in the relative perirenal adipose tissue (PAT) mass at 21d. Plasma insulin concentrations during the first 24h after birth were strongly correlated with size of perirenal adipocytes at 21d ( $r^2=0.57$ ,  $P<0.0002$ ). PPAR $\gamma$  ( $P<0.05$ ) and leptin ( $P<0.001$ ) expression in PAT was lower in LBW compared with ABW male lambs. There were also significant relationships between both PPAR $\gamma$  and leptin expression in PAT and birth weight in males. In contrast, birth weight did not influence PPAR $\gamma$  and leptin expression in female lambs. Female lambs had lower plasma insulin concentrations and higher relative PAT mass ( $P<0.05$ ) than males, and in females PPAR $\gamma$  expression was directly related to mean plasma insulin ( $r^2=0.41$ ,  $P<0.05$ ) and the size of perirenal adipocytes ( $r^2=0.64$ ,  $P<0.01$ ). In females, leptin expression in PAT was also related to the size of perirenal adipocytes ( $r^2=0.50$ ,  $P<0.05$ ). Plasma leptin was not different between LBW and ABW lambs, or between male and female lambs, and was not related to any measure of fat mass at 21d. There was no effect of birth weight or gender on RXR $\alpha$ , adiponectin, LPL or G3PDH expression in perirenal fat. **Conclusions:** There are differences in the effects of birth weight on adipose gene expression in male and female lambs. We postulate that the reduced expression of PPAR $\gamma$  and leptin in perirenal fat of LBW male lambs may be related to an impaired insulin sensitivity, when compared with female lambs. The differential effect of birth weight on adipocyte gene expression in male and female lambs may be important in gender specific programming of an increase in visceral fat mass in adult life.

## A23

### THE IMPACT OF MATERNAL OVERNUTRITION IN LATE GESTATION AND GENDER ON FAT DEPOSITION AND FEEDING BEHAVIOUR IN THE POSTNATAL LAMB

**BS Muhlhausler**<sup>1</sup>, JA Duffield<sup>1</sup>, IC McMillen<sup>1</sup>

<sup>1</sup>Research Centre for the Early Origins of Adult Health, The University of Adelaide, Australia  
beverly.muhlhausler@adelaide.edu.au

**Aims:** Epidemiological studies have demonstrated that infants of diabetic mothers, who are exposed to an elevated nutrient supply during development, are at increased risk of obesity in later life. This has led to the suggestion that prenatal overnutrition is an important determinant of appetite and body composition in later life. In the present study, we have determined the effect of an increased maternal nutrient supply in late gestation on growth, feeding behaviour and fat deposition in the postnatal lamb.

**Methods:** From 115 d gestation until delivery pregnant ewes were fed a diet which provided either 100% (control, n=12; 6 Males, 6 Females) or ~150% (well fed (WF), n=11 4 Males, 7 Females) of maintenance energy requirements (MER). All feed refusals were weighed and recorded daily. Lambs were delivered spontaneously and birthweight was recorded within 6 h of birth. Feed intake was determined by a two-hour weigh-suckle-weigh (WSW) protocol in each lamb on day (d) 2,5,8,11,14, 20 and 23 of postnatal life, and total weight gain of lambs during the 2 h WSW protocol was used to calculate feed intake during this period. Total body weight and mass of the perirenal, subcutaneous and omental fat depots were recorded in these lambs at 30 d of age. Differences between control and WF groups and between male and female lambs were determined by two-way ANOVA.

**Results:** Maternal nutrient intake between 115 d gestation and delivery was higher in the WF group ( $129 \pm 4$  % vs  $90 \pm 2$  % MER,  $P < 0.001$ ) and was positively correlated with the relative mass of both subcutaneous ( $R = 0.60$ ;  $P < 0.05$ ,  $n = 23$ ) and omental ( $R = 0.66$ ;  $P < 0.04$ ,  $n = 11$ ) adipose tissue when data from all lambs were combined. The relative mass of subcutaneous fat was higher in lambs of WF ewes compared to the control group independent of gender ( $34.9 \pm 4.7$  g/kg vs  $22.8 \pm 3.3$  g/kg;  $P < 0.05$ ). Total weight gain (g), a measure of feed intake during the 2 h WSW protocol, and percentage weight gain (% start weight) during the WSW protocol were each higher ( $P < 0.05$ ) in lambs of WF ewes during the first week of postnatal life independent of gender. There was no difference in the relative mass of the perirenal or omental fat depots between control and WF groups. Perirenal fat mass was, however, significantly higher ( $P < 0.01$ ) in females compared to males in both birthweight groups. Birthweight and 30d weight were also not different between lambs from control or WF ewes.

**Conclusion:** We have therefore demonstrated that increasing maternal nutrient intake in late gestation programs increased fat deposition in early postnatal life, and that this is directly related to the extent of overnutrition in late pregnancy.

# A24

## Effect of Periconceptional Undernutrition on Insulin Sensitivity at 65 days Gestation in Singleton Bearing Pregnant Ewes

**Jaquiery AL**, Oliver MH, Buckley A, Harding JE

Liggins Institute, Faculty of Medicine and Health Sciences, University of Auckland, Private Bag 92019, Auckland New Zealand

**Background** Periconceptional undernutrition alters fetal growth, metabolism and endocrine status in late gestation. A possible mechanism underlying these effects could be nutritionally induced perturbation of maternal physiological adaptation to pregnancy. One such adaptation involves the development of maternal insulin resistance, allowing more nutrients to be available for fetal growth.

**Aims** To determine the effects of periconceptional undernutrition on maternal insulin sensitivity in mid-gestation.

**Methods** Ewes were randomly assigned to one of four nutritional groups: Control (N); Undernourished from 61 days before to 30 days after mating (UN-60-30d); Undernourished from 61 days before until mating (UN-60-0d); Undernourished from 2 days before to 30 days after mating (UN-2-30d). Control ewes were fed to maintain body weight $\pm$ 5%. Undernourished ewes were fed to achieve and maintain 15% weight loss after an initial two day fast. At 65 days of gestation, singleton-bearing ewes underwent assessment of insulin sensitivity using a hyperinsulinaemic-euglycaemic clamp (HEC). Insulin sensitivity was calculated as the ratio of steady-state glucose infusion rate (mg glucose/kg/min) to steady-state plasma insulin concentration ( $\mu$ U/ml). Groups were compared using factorial ANOVA with Fishers post-hoc correction for multiple comparisons.

**Results** Twenty-seven singleton-bearing ewes underwent HEC. Two were excluded from analysis as steady-state plasma insulin concentrations were not achieved.

	Control	UN-60-30d	UN-60-0d	UN-2-30d
n	10	5	4	6
weight (kg)	72.2 $\pm$ 1.8	61.5 $\pm$ 4.8	69.8 $\pm$ 3.8	68.2 $\pm$ 3.5
Basal plasma glucose (mmol/l)	2.5 $\pm$ 0.2	2.3 $\pm$ 0.1	2.3 $\pm$ 0.1	2.5 $\pm$ 0.0
Basal plasma insulin ( $\mu$ U/ml)	1.2 $\pm$ 0.2	1.2 $\pm$ 0.3	0.8 $\pm$ 0.3	1.6 $\pm$ 0.4
Insulin sensitivity (mg.l/ $\mu$ U/kg/min)	2.61 $\pm$ 0.39	4.36 $\pm$ 0.92	4.92 $\pm$ 0.57	2.63 $\pm$ 0.37

Values are mean $\pm$ SE

Mean weight at the time of HEC was significantly lower in the UN-60-30d group than in the N group ( $p=.017$ ). There were no differences between nutritional groups in basal plasma glucose or insulin concentrations. Ewes in groups UN-60-30d and UN-60-0d were significantly more insulin sensitive than control (both  $p<.03$ ) and UN-2-30d ewes (both  $p<.05$ ).

**Conclusions** Prolonged undernutrition prior to conception affects insulin sensitivity at 65 days gestation in singleton bearing ewes, even after 35 (UN-60-30d) or 65 (UN-60-0d) days of refeeding. This may affect the availability of fetal nutrient supply. Conversely, undernutrition for 30 days from the time of mating had no effect on maternal insulin sensitivity at 65 days, suggesting that either the timing (pre- vs post-conception) or duration (60 days vs 30 days) of periconceptional undernutrition is a key factor influencing adaptation of glucose and insulin metabolism in pregnancy.

## A25

### DIFFERENTIAL EFFECTS OF GESTATIONAL AGE AND PLACENTAL RESTRICTION ON THE PROPORTIONS OF SPECIFIC CORTICOTROPH SUBPOPULATIONS IN THE FETAL SHEEP PITUITARY DURING LATE GESTATION

**Farrand K**, McMillen IC, Schwartz J

Centre for the Early Origins of Adult Health, Discipline of Physiology, School of Molecular & Biomedical Sciences, Faculty of Science, University of Adelaide, Adelaide, Australia

**Background:** Placental insufficiency restricts fetal substrate supply inducing fetal hypoxia and hypoglycaemia. As a result, the fetal hypothalamic-pituitary adrenal axis has been shown to undergo particular adaptations. In sheep, experimental induction of placental restriction results in higher circulating levels of fetal cortisol in late gestation (term ~147 days gestation (d)) in the absence of a concomitant increase in fetal plasma adrenocorticotrophic hormone (ACTH) concentrations. In addition, there is a decrease in fetal pituitary pro-opiomelanocortin (POMC) mRNA levels following placental insufficiency but an increase in the basal secretion of ACTH *in vitro* from a subset of corticotroph cells which are unresponsive to corticotrophin releasing hormone (CRH).

**Aims:** To investigate whether the relative proportions of corticotrophs that express POMC, ACTH and the CRH receptor, R1, are altered in the pituitary of fetuses subjected to chronic substrate restriction. **Methods:** Placental restriction (PR) was induced by removal of the majority of the placental attachment sites in five ewes before mating. Pituitaries were collected from control and PR fetal sheep at 140 d (control n=4, PR n=4) and 144 d (control n=6, PR n=4). Pituitary sections were labelled with antisera raised against POMC, ACTH and R1.

**Results:** The proportion of pituitary cells expressing POMC and/or ACTH (ie corticotrophs) decreased ( $p < 0.05$ ) between 140 d ( $15 \pm 2\%$ ) and 144 d ( $11 \pm 1\%$ ) in both the control and PR groups. Three major subpopulations of corticotrophs were identified which expressed either POMC+ACTH+ R1, ACTH+R1 or POMC only. The proportion of corticotrophs co-expressing POMC+ACTH+R1 decreased ( $p < 0.05$ ) between 140d (control  $60 \pm 1\%$  PR  $66 \pm 4\%$ ) and 144 d in both groups (control  $45 \pm 2\%$ , PR  $56 \pm 6\%$ ). However, there was a significantly greater ( $p < 0.05$ ) proportion of corticotrophs which co-expressed POMC+ACTH+R1 in the pituitary of the PR group when compared to controls at both gestational ages.

**Conclusions:** Subpopulations of corticotrophs can be identified in the fetal sheep pituitary based on the differential expression of POMC, ACTH and R1. Gestational age and placental restriction each significantly impact on the proportions of corticotrophs in specific subpopulations. Exposure of the sheep fetus to chronic substrate restriction shifts the fetal pituitary corticotroph population towards a conservation of the subpopulation of corticotrophs which co-express POMC, ACTH and R1.

## A26

### **Inadequate compensatory increase in $\beta$ -cell mass and insulin secretion in young adult males following placental restriction of fetal growth in sheep.**

**KL Gattford**, P Standen\*, WM Foong\*, C Roberts, JA Owens  
Department of Obstetrics and Gynaecology, University of Adelaide

It is now recognised that Type 2 diabetes occurs when insulin secretion fails to increase sufficiently to compensate for insulin resistance. In most individuals,  $\beta$ -cell mass and insulin secretion adapt to maintain appropriate insulin action, but in others, this compensation is inadequate and fails, resulting in diabetes. The origins of individual variability in  $\beta$ -cell capacity to compensate are unknown. Intrauterine growth restriction (IUGR) and small size at birth are risk factors for development of impaired glucose tolerance and Type 2 diabetes in adulthood. This suggests that the prenatal environment that restricts fetal growth may impair the capacity of the pancreas to increase  $\beta$ -cell mass and insulin secretion (plasticity) when required.

We have therefore induced IUGR in sheep by surgically removing most implantation sites from the non-pregnant uterus (placental restriction, PR). This restricts placental implantation, growth and function, and reduces birth weight by ~25%. Lambs born to unoperated control ewes and to PR ewes were studied from birth to 18 months of age. We independently measured insulin secretion (IVGTT) and insulin sensitivity (hyperinsulinaemic euglycaemic clamp) in young adult sheep at 12 months of age. We then calculated their insulin disposition index, a measure of insulin secretion relative to sensitivity or demand. Pancreas was dissected and weighed at 18 months of age, and fixed in 4% paraformaldehyde. Sections (5 $\mu$ m) were immunolabelled for insulin and  $\beta$ -cell volume density, islet numerical density and islet  $\beta$ -cell number determined.

PR increased pancreas weight as a proportion of liveweight in males (CON: 0.102  $\pm$  0.003%, PR: 0.119  $\pm$  0.004%, P=0.05) and tended to overall (CON: 0.101  $\pm$  0.001%, PR: 0.110  $\pm$  0.002%, P=0.054). In males, PR increased  $\beta$ -cell volume density (CON: 1.8  $\pm$  0.2%, PR: 3.6  $\pm$  0.5%, P=0.007) and  $\beta$ -cell mass (CON: 1.20  $\pm$  0.15 g, PR: 2.47  $\pm$  0.27 g, P=0.005) and  $\beta$ -cell mass tended to correlate negatively with birth weight ( $r$ =-0.54, P=0.1, n=10). PR tended to increase the numerical density of islets in males (x 1.7) (CON: 17.1  $\pm$  2.4, PR: 28.5  $\pm$  5.5, P=0.08), and increased that of small islets (3 or less  $\beta$ -cells) even more so (x 3.4) (CON: 1.6  $\pm$  0.5, PR: 5.5  $\pm$  1.8, P=0.04). As small islets may represent de novo generation of  $\beta$ -cells or neogenesis, the possibility that PR increases  $\beta$ -cell mass in part via this process requires further investigation. Despite increased  $\beta$ -cell mass, basal ( $r$ =0.68, P=0.015, n=12) and glucose-stimulated ( $r$ =0.57, P=0.054, n=12) insulin disposition fell with decreasing size at birth in males. In preliminary analyses, basal insulin disposition divided by  $\beta$ -cell mass was positively related to birth weight in males ( $r$ =0.78, P=0.038, n=7), which suggests that  $\beta$ -cell capacity to secrete insulin *in vivo* is impaired.

These findings suggest that the plasticity of  $\beta$ -cell mass and/ or individual  $\beta$ -cell insulin secretory capacity are impaired or insufficient to compensate for the insulin resistance that develops in the young adult male sheep that grew poorly before birth. To determine if PR impairs the plasticity of  $\beta$ -cell mass, it will be necessary to subject control and PR sheep to a similar demand for insulin. We plan to do this in adolescent sheep, prior to development of insulin resistance following PR. Similarly, direct quantitation of  $\beta$ -cell insulin secretory capacity *in vitro* will further define the consequences for this of PR.

(\* Wai-Mee Foong and Prue Standen assisted with this project, as Summer Research Scholars).

## A27

### Tissue specific expression of Suppressor of Cytokine Signalling (SOCS)-3 during fetal development

S Gentili, MJ Waters\*, IC McMillen

Research Centre for the Early Origins of Adult Health, Discipline of Physiology, School of Molecular and Biomedical Science, The University of Adelaide, Adelaide, South Australia, Australia, 5005

\*Institute for Molecular Bioscience, The University of Queensland, Brisbane, Queensland, Australia, 4067

In postnatal life, growth hormone (GH) and prolactin (PRL) act on a range of tissues through the intracellular JAK/STAT signalling pathway, which in turn is inhibited by SOCS-3. Whilst SOCS-3 plays a central role in the regulation of tissue growth in postnatal life, the role of SOCS-3 before birth is unknown. The aim of this study, therefore was to quantify SOCS-3 expression in a range of tissues in the sheep fetus throughout gestation.

Sixty pregnant ewes were used in this study at 54d, 95-110d, 124-125d 131-133d and 141-145d gestation. Fetal organs were removed, weighed, and samples frozen for RNA extraction. SOCS-3 and  $\beta$ -actin mRNA expression were measured by RT-PCR.

There was a significant increase ( $P < 0.005$ ) in SOCS-3 expression between 54d gestation and 141-145d gestation in the liver of fetal sheep. In contrast, SOCS-3 expression significantly decreased ( $P < 0.005$ ) between 54d and 141-144d gestation in the fetal adrenal gland and between 90-91d gestation and 140-145d gestation in the perirenal adipose tissue.

We have therefore demonstrated that there is a differential pattern of SOCS-3 expression in fetal tissues with increasing gestational age. In the liver SOCS-3 expression is upregulated by cytokine signalling in late gestation whereas in the fetal adrenal and adipose tissue there is suppression of cytokine stimulation of SOCS-3 expression in late gestation.

## SURFACTANT PHOSPHOLIPID SPECIES: CHANGES IN ENDOTRACHEAL ASPIRATES OF NEONATES WHO DEVELOP BRONCHOPULMONARY DYSPLASIA

**D. A. Todd**<sup>§\*</sup>, H. Clark<sup>\*\*</sup>, J. Calvert<sup>\*\*</sup>, A. George<sup>\*</sup> & A D. Postle<sup>\*</sup>

Neonatal Intensive Care (NICU) <sup>§</sup>, Westmead Hospital, Westmead, Sydney, NSW Australia, Child Health<sup>\*</sup>, Southampton General Hospital, Southampton, Hampshire, England and NICU<sup>\*\*</sup> John Radcliffe Hospital, Oxford, England.

David\_Todd@wsahs.nsw.gov.au

**Introduction:** Bronchopulmonary Dysplasia (BPD) continues to be a major morbidity in premature infants. Much is known about pulmonary surfactant in preterm infants with hyaline membrane disease, but there is less information about changes to airway and alveolar phospholipid compositions during disease progression to BPD (1, 2). With improvements in methodology however, any changes in phospholipid molecular species may now be identified (3).

**Aims:** To quantify changes to phosphatidylcholine (PC) molecular species in the endotracheal aspirates (ETA) of neonates who develop BPD (oxygen requirement at 36 weeks post conceptional age).

**Methods:** Endotracheal aspirates were collected from neonates in the NICU at John Radcliffe, Oxford who required routine suctioning of endotracheal tubes until 28 days and cell-free supernatants were stored at  $-80^{\circ}\text{C}$  until analysis. Following organic extraction of the phospholipids, PC molecular species compositions of ETA's were determined by electrospray ionisation mass spectrometry (3). The results of the PC molecular species were grouped according to the age of the infants (1-3, 4-7, 14-20 & 21-28 days). Results over time for each PC molecular species were compared using the Linear Mixed Effects Model (S-plus: 6.1). The study was approved by the local research ethics committee.

**Results:** From 19/1/01 to 22/3/02, 10 (24.4%) of 41 neonates (gestational age 23-32 weeks) developed BPD. In this group of intubated infants, 3/10 (30%) neonates <27 wks gestation died and 6/10 (60%) developed BPD. Only 4/16 (25%) neonates 27-29 wks gestation and no neonates 30-32 wks developed BPD. The PC molecular species in ETA samples from neonates (n=12) who developed BPD &/or died (at >3 weeks of age) were characterized by decreased dipalmitoyl PC (DPPC or PC 16:0/16:0, 48 to 44 mole %), with a corresponding significant increase ( $p<0.001$ ) in the palmitoylinoyleoyl PC (PC16:0/18:2, diagnostic of plasma, 3 to 11.4 mole %), and no change of palmitoylloyleoyl PC (PC16:0/18:1, which is enriched in cell membranes).

**Conclusion:** Analysis of the PC profile of ETA samples from neonates with BPD suggested an influx of plasma phospholipid, consistent with impairment to the alveolar capillary barrier. Despite the known cellular increase in the lungs during BPD, the cellular PC components remain unchanged. We speculate that this alteration in the PC profile with a lowering of DPPC, an increase in PC 16:0/18:2 and damage to the alveolar capillary membrane may contribute to the increased ventilation requirements and the subsequent development of BPD. More studies are required to confirm these changes.

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## A29

### ACUTE MATERNAL ALCOHOL ADMINISTRATION CAN INDUCE WHITE MATTER INJURY IN THE PRETERM OVINE BRAIN

**P. Dalitz**<sup>1</sup>, J. Duncan<sup>2</sup>, P. Henschke<sup>3</sup>, S Rees<sup>2</sup>, M. Cock<sup>1</sup>, S. Osborn<sup>1</sup>, R. Harding<sup>1</sup>.  
<sup>1</sup>Physiology, Monash Univ. VIC 3800; <sup>2</sup>Anatomy & Cell Biology, Univ. Melbourne, VIC 3100 and <sup>3</sup>Royal Women's Hospital, Melbourne, VIC, Australia, 3053.

**AIMS:** Recent clinical and animal evidence suggests that 'binge' patterns of maternal alcohol consumption may be particularly harmful to the fetal brain. Using an ovine model of repeated, acute, maternal EtOH consumption, we have examined effects on the developing fetal cerebral and cerebellar white matter (WM) and potential mechanisms of altered fetal neuropathology.

**METHODS:** EtOH (1g/kg of maternal weight) was administered to 8 twin-bearing, chronically catheterised ewes for 1 hour on 3 consecutive days at 0.8 of gestation; controls (n=6) received saline. Two days after the final infusion, brains were processed for structural or biochemical analysis. Fetal brains were assessed for alterations in gross morphology (H&E), astrocytes (GFAP), microglia and macrophages (Lectin), axons (Bielschowsky), oligodendrocytes (CNPase) and apoptosis (TUNEL). We examined the incidence of astrogliosis and apoptosis by quantifying GFAP- and TUNEL- positive cells (cells/mm<sup>2</sup>) respectively. Cell numbers were quantified in the peri-ventricular-, deep- and sub-cortical- WM in the frontal, parietal, temporal and occipital lobes. Biochemical analysis of the fetal brain examined lipid hydroperoxide (LPO) levels as an indicator of oxidative stress. Fetal plasma, collected prior to EtOH infusion and at 1h, 2h, 3h and 6h on each experimental day, was analyzed for levels of pro-inflammatory cytokines (tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ ) and interleukin-6 (IL-6)) by ELISA.

**RESULTS:** EtOH in the mother and fetus reached maximal levels of 0.11 $\pm$ 0.01 g/dL 1h after the infusions. At autopsy, mean body weights of EtOH fetuses were ~400g lower than controls (p=0.02) and there was evidence of brain sparing. In 3 of the 8 EtOH exposed fetuses, we observed areas of subcortical WM injury ranging from 1.6-11.5% of the cross-sectional area of WM; injury occurred in the frontal, parietal or occipital lobes. In areas of damage, lectin-, GFAP- and TUNEL- positive cells were observed. Lesser alterations in white matter were observed in 2 other fetuses. Two EtOH exposed fetuses (one displaying subcortical WM injury and one that did not) had WM gliosis in the cerebellum. Disruption to axons within the cerebral and cerebellar WM was revealed by Bielschowsky staining. No brain injury was observed in control fetuses. Quantitative analysis revealed a significant increase in apoptotic cell number (cells/mm<sup>2</sup>) in EtOH fetal brains in comparison to controls (p=0.005). The number(s) of apoptotic cells were significantly increased throughout the peri-ventricular-, deep- and sub-cortical- WM in frontal, parietal, temporal and occipital lobes. There was a significant increase in the number of GFAP positive cells (cells/mm<sup>2</sup>) in the brains of EtOH exposed fetuses (p=0.05) in comparison to controls.

LPO levels tended to be elevated (not significant) in the WM, corpus callosum, hippocampus, brainstem, thalamus and spinal cord of EtOH fetuses compared to controls. In a related study of acute fetal EtOH exposure, LPO levels were elevated in fetal arterial plasma of EtOH-exposed fetuses following maternal EtOH infusions. Levels of pro-inflammatory cytokines were not elevated in fetal plasma samples at any stage in either the acute or acute, episodic study.

**CONCLUSIONS:** Acute, episodic ethanol exposure can result in WM injury in the cerebral hemispheres and cerebellum in the immature fetal brain. Furthermore, this 'binge' pattern of EtOH exposure can significantly increase the incidence of global apoptosis and astrogliosis in the fetal brain. The mechanisms for white matter injury remain unknown: they are unlikely to be a result of cerebral hypoxia but could involve oxidative stress.

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# A30

## THE EFFECT OF ANTENATAL ALCOHOL AND FISH OIL EXPOSURE ON CARDIOVASCULAR DEVELOPMENT IN RATS

Lucy Liu, Sarah Richards, Peter Howe, Jeff Schwartz.  
Discipline of Physiology, School of Molecular and Biomedical Science  
University of Adelaide, Adelaide, SA.

Prenatal alcohol exposure affects cardiovascular development. In previous studies from this lab, we found binge exposure to alcohol on day 8 of gestation (B8) in rats is associated with cardiac hypertrophy and an increased blood pressure (BP) response to angiotensin II. In this study, we focus further on the effect of alcohol on BP in B8 rats and whether exposure to fish oil (high n-3) counters the effect of alcohol.

Pregnant Sprague Dawley rats were given either fish (FO) or canola (CO) oil (1g/d) by feeding tube from day 4 to day 21 of gestation (term: 22days). On day 8 the rats were fed 5g/kg ethanol (Alc; 50% in water) or an isocaloric solution of sucrose (Su) in water (N=3 or 4 litters per treatment group). At birth, all pups were measured. Pups in excess of 8 were dissected for organ measurement. The 8 pups were returned to their dams and were weaned at 3 weeks. At 10 weeks, we measured BP by the tail-cuff method. Five independent measurements were made for each rat, and the data were not analysed until BP of all rats had been measured.

Data are summarised in the table below. As in previous studies, alcohol induced no difference of individual pup weight at birth. There were significantly fewer pups per litter in the B8 group and therefore lower total litter weight ( $p<0.05$ ) in B8 litters. This effect was reversed by fish oil.

There were no significant effects of either treatment on heart or kidney weight (not shown). At 10 weeks, B8 rats had higher systolic (s) BP ( $p<0.005$  for males,  $p<0.01$  for females), but fish oil had no effect on the BP change. Interestingly, the fish oil treatment is associated with lower left ventricular weight (LV/BW) corrected for body weight in female offspring ( $p<0.01$ ) independent of the effect of alcohol. Results for all pups in a litter were averaged to yield  $N = 1$  and analysed as the means of litters. Data are presented as mean  $\pm$  sem

Treatment	No. of litters	Average pup wt. (g)	Pups/litter	Total litter Wt. (g)	Male sBP	Female sBP	Female LV/BW (mg/g)
CO + Su	4	6.6 $\pm$ 0.3	14 $\pm$ 0	90.7 $\pm$ 2.2	124.1 $\pm$ 1.5	119.0 $\pm$ 1.2	2.7 $\pm$ 0.3
CO + Alc	3	7.0 $\pm$ 0.7	11 $\pm$ 0	74.3 $\pm$ 4.7	128.1 $\pm$ 2.3	122.2 $\pm$ 0.7	2.5 $\pm$ 0.3
FO + Alc	3	7.0 $\pm$ 0.4	12 $\pm$ 1	85.0 $\pm$ 2.3	129.2 $\pm$ 1.6	121.4 $\pm$ 0.3	2.4 $\pm$ 0.2
FO + Su	4	6.4 $\pm$ 0.1	12 $\pm$ 1	74.4 $\pm$ 4.5	125.7 $\pm$ 1.1	121.0 $\pm$ 0.6	2.4 $\pm$ 0.2

Taken together, these data are consistent with a single fetal exposure to alcohol being able to alter fetal developmental outcome, such that postnatal cardiovascular regulation is altered. The difference in BP, as measured by tail cuff sphygmomanometry, may represent an effect of alcohol on resting BP or on the BP during stress. Either way, the results suggest that antenatal exposure to alcohol alters regulatory mechanisms. The finding of decreased relative left ventricular weight in females treated antenatally with fish oil is consistent with a protective effect of fish oil on vascular resistance.

# A31

## CHARACTERIZATION AND EXPRESSION OF GENES ACTIVATED BY VENTILATOR-INDUCED LUNG INJURY

**Alison Thiel**, Megan Wallace, Megan Probyn, Richard Harding, Colin Morley<sup>1</sup> & Stuart Hooper. Department of Physiology, Monash University and <sup>1</sup>Neonatal Services, Royal Women's Hospital, Melbourne, Australia. alison.thiel@med.monash.edu.au

**Introduction:** Infants born very pre-term (<30 weeks) are at increased risk of developing chronic lung disease (CLD) as their lungs are structurally immature, surfactant deficient and incompliant. These infants usually require assisted ventilation from birth which can injure their lungs, induce an inflammation response and arrest lung development, which together characterize CLD. To improve the outcome for these infants, it is important to identify resuscitation and ventilation strategies that minimize lung injury as well as identify infants with injured lungs during the immediate inflammatory phase of the injury. Although we have been unable to show an increase in the inflammatory markers NFκB, TNFα and TGFβ<sub>1</sub> within 2 hours following the onset of artificial ventilation in preterm lambs, evidence of lung damage (i.e. the presence of neutrophils and RBC's in the airways) has been observed. However, the inflammatory pathways activated in response to ventilator-induced acute lung injury are largely unknown and there are no known markers that reliably predict the onset of lung injury. We have recently cloned a gene, IRF2-BP2 from the lung of fetal sheep and have shown that its expression is increased within 2hs of lung aeration. Thus, our **aim** was to characterize the changes in expression of this gene in the lung of prematurely delivered lambs in response to a ventilation strategy that would induce lung injury. The protein encoded by this gene, and closely related proteins, play an important role in the activation and suppression of genes involved in cell cycle progression and inflammation. Thus, we **hypothesized** that it may play a role in the initiation of the inflammatory cascade associated with ventilator-induced lung injury.

**Methods:** At 126d (term~147d), fetal sheep were exteriorised by caesarian section and a carotid artery and jugular vein were catheterised before the fetus was intubated and delivered. Lambs were then mechanically ventilated for 15 mins at a V<sub>T</sub> of 20ml/kg in the absence of PEEP; this was expected to cause lung injury. After 15 mins, the lambs were ventilated at a V<sub>T</sub> of 5ml/kg and a PEEP of 4 cmH<sub>2</sub>O for 15, 30, 60 and 120 mins (n=4 for each time point).

**Results:** Three transcripts encoding IRF2-BP2 were detectable by northern blot (7.25kb, 4.4kb and 3.25kbs). The 3.25kb transcript was the most abundant and its mRNA levels were expressed as a proportion of mRNA levels of 125d control fetal lung tissue. The mRNA levels of the 4.4kb transcript were increased from 100.0 ± 14.7% in control fetal lung tissue to 214.3 ± 15.3% at 15mins, 247 ± 23.7% at 30mins, 231.6 ± 5.9% at 60 mins and 154.6 ± 29.5% at 120 mins after the injurious ventilation period. *In-situ* hybridization was used to determine the cell types expressing IRF2-BP2 30 minutes after the initial injurious ventilation period. The expression of IRF2-BP2 in control sections was confined to cells located around the airways, while at 30 minutes, IRF2-BP2 expression was uniformly increased across all cells.

**Conclusions:** The mRNA levels for IRF2-BP2 are significantly up-regulated immediately after an injurious ventilation strategy with the most significant increase at 30 minutes. IRF2-BP2 expression was localized to small airways in control lung tissue but expression in the peri-alveolar interstitial tissue region was markedly elevated in response to injury. Although further investigation is required to determine the potential role of this gene in the inflammatory response to lung injury, it could serve as an early marker of marker of injury.

## Characterization of Sex-Specific Differences in Preterm Neonatal Cardiovascular Adaptation – A Study Proposal

**Stark MJ<sup>1</sup>**, Wright IMR<sup>2</sup>, Hirst J<sup>3</sup>, Walker DW<sup>3</sup>, Clifton VL<sup>1</sup>

Mothers and Babies Research Centre, Hunter Medical Research Institute University of Newcastle<sup>1</sup>, and Kaleidoscope Neonatal Intensive Care Unit John Hunter Children's Hospital<sup>2</sup>, Newcastle, NSW, and The Department of Physiology, Monash University, Clayton, Victoria, Australia

**Background:** Male sex is one of the best predictors of death in preterm infants. In normal pregnancy very low concentrations of cortisol are present in the fetal compartment until late in gestation. We have demonstrated fetal exposure to elevated cortisol in response to a maternal stressor results in a sex-specific difference in fetal HPA response and downstream effects on pathways regulated by glucocorticoids [4, 5]. The female fetus appears to be more sensitive to changes in glucocorticoid concentration [7] slowing growth as an adaptive response to potential intra-uterine compromise. Glucocorticoids have a key role in cardiovascular adaptation influencing microvascular function through maintenance of vascular tone and potentially central blood pressure control via GABAergic pathways. Microvascular vasodilatory capacity is mediated by several vasoactive substances, including nitric oxide (NO) and carbon monoxide (CO) which both increase smooth muscle cGMP. Glucocorticoids have an inhibitory effect on these pathways. Our group has previously demonstrated a sex-specific vasodilatory response to CRH acting via the NO pathway, and we have pilot data that demonstrates a sex-specific difference in CO production between male and female neonates that may increase the risk of males developing cardiac dysfunction. We hypothesise that there is a sex-specific difference in the response to glucocorticoids in the prenatal and immediate post-natal period in the male and female neonate. This difference may significantly contribute to microvascular control and cardiovascular adaptation in neonatal life resulting in the male neonate being more susceptible to cardiac dysfunction than the female.

**Aims:** To characterise the sex-specific differences in:

1. Adrenal status at the time of delivery in male and female neonates in relation to the time of exposure to betamethasone and gestational age.
2. Microvascular and macrovascular function in relation to adrenal status within an individual over the first 7 days of life.
3. Markers of cardiovascular dysfunction by measuring adrenomedullin, and endothelin-1 in the first 7 days of life and relate these to neonatal microvascular function, morbidity and mortality.

**Experimental Protocol:** Two groups of neonates, <28 and 28-36 completed weeks gestation will be analysed by gender. Cord blood and daily neonatal plasma samples for days 1-7 of life will be collected and cortisol, DHEAS, pregnanolone, and allopregnanolone assayed. Neonatal urine will be collected for each 24-hour period from day 1-7 for analysis of cortisol and adrenal steroid metabolites by gas chromatography mass spectroscopy (GCMS). Laser Doppler assessment of the peripheral vasculature will be performed on days 1, 3, 5, and 7 and indirect markers of microvascular function determined by analysis of urinary NO metabolites, plasma cGMP, and carboxyhaemoglobin. Echocardiographic assessment will be performed on days 1-7 documenting superior vena caval flow, cardiac function, left and right ventricular outputs and correlated with the laser Doppler studies, markers of microvascular function and the vasoactive mediators ADM and ET-1.

## A33

### Measurement of cardiovascular variables during sleep in infants aged 2-4 months: effects of sleeping position and sleep state

**Yiallourou SR**, Ramsden A, Walker AM and Horne RSC, Ritchie Centre for Baby Health Research, Monash Institute of Medical Research, Monash University, Melbourne.

**Background:** Impaired autonomic cardiovascular control has been implicated as one of the underlying mechanisms for Sudden Infant Death Syndrome (SIDS). To date there is limited knowledge of cardiovascular control during sleep in infants due to the inability to measure variables such as blood pressure and blood flow non-invasively. New techniques are now available and these critical variables can now be recorded.

**Aim:** To define the effects of prone sleeping, the major risk factor for SIDS, on peripheral blood flow (PBF), blood pressure (BP) and heart rate (HR) during both active sleep (AS) and quiet sleep (QS) in infants at 2-4 mo of age when they are most vulnerable to SIDS.

**Methods:** Five healthy term infants (38-42 wks) with normal birth weights ( $3.62 \pm 0.06$  kg) and Apgar scores averaging 9 and 9 at 1 and 5 minutes respectively were studied at 2-4 mo of age. Daytime polysomnography was performed following the infants normal sleep patterns. To determine sleep state electroencephalogram (EEG), submental electromyogram (EMG), electro-oculogram (EOG), heart rate (HR), abdominal and thoracic respiratory movements, oxygen saturation and behavioural patterns were recorded. Measurements of BP were recorded in 1 minute epochs using a photoplethysmographic cuff placed around the infant's wrist (Finometer<sup>TM</sup>, FMS, The Netherlands). Peripheral blood flow was measured continuously using a Laser Doppler Flowmeter (ADInstruments, Sydney, Australia) attached to the infants forearm. Multiple measurements (n=4) were taken during both AS and QS in both the prone and supine positions.

**Data analysis:** Arterial pressure data for mean (MAP), systolic (SAP) and diastolic (DAP); peripheral blood flow data for mean (PBFm), maximum (PBFmax) and minimum (PBFmin) and HR were obtained on a beat by beat basis by peak detection in 1 min epochs. BP, PBF and HR were compared between sleep states and sleeping positions with two-way repeated measures ANOVA. Data are expressed as mean  $\pm$  sem with  $p < 0.5$  considered as statistically significant.

**Results:** Mean values for BP, PBF, and HR are summarised below. HR was higher in the prone position compared to the supine position in both AS and QS. There was no effect of sleep states or sleeping positions on BP or PBF.

	MAP (mmHg)	SAP (mmHg)	DAP (mmHg)	mean PBF (arbitrary units)	max PBF (arbitrary units)	min PBF (arbitrary units)	HR (bpm)
qs-supine	72 $\pm$ 6	81 $\pm$ 6	65 $\pm$ 6	10 $\pm$ 3	12 $\pm$ 4	10 $\pm$ 3	124 $\pm$ 3*
as-supine	74 $\pm$ 9	89 $\pm$ 8	67 $\pm$ 9	12 $\pm$ 4	13 $\pm$ 5	11 $\pm$ 4	126 $\pm$ 2*
qs-prone	71 $\pm$ 7	84 $\pm$ 9	64 $\pm$ 7	10 $\pm$ 2	12 $\pm$ 3	10 $\pm$ 2	130 $\pm$ 2
as-prone	72 $\pm$ 7	86 $\pm$ 7	65 $\pm$ 8	11 $\pm$ 3	13 $\pm$ 3	11 $\pm$ 3	134 $\pm$ 3

\* $p < 0.05$  between prone and supine sleeping positions

**Conclusion:** This is the first study to non-invasively measure HR, BP and PBF simultaneously in sleeping infants. These preliminary data show that HR was increased in the prone position, which is supported by previously reported data. Although no other cardiovascular effects on sleep state or sleeping position at 2-4 mo of age were found, further analysis of our complete data set are required to confirm this.

## VARIABILITY OF THE HYPOXIC VENTILATORY RESPONSE IN SLEEPING INFANTS

Richardson HL<sup>1</sup>, Parslow PM<sup>1</sup>, Walker AM<sup>1</sup>, Harding R<sup>2</sup>, Horne RSC<sup>1</sup>

<sup>1</sup>Ritchie Centre for Baby Health Research, Monash Institute for Medical Research and  
<sup>2</sup>Department of Physiology, Monash University, Melbourne, Australia

**Background:** Previous studies of infants exposed to hypoxia have reported a biphasic ventilatory response consisting of an initial increase followed by a reduction in ventilation. Over time this infantile response matures, adopting a monophasic appearance, although the age at which this change occurs remains controversial. Despite numerous studies concerning the hypoxic ventilatory response (HVR) of human infants and its maturation, previous studies have primarily been conducted in quiet sleep (QS) alone and furthermore, few studies have made repeated measurements at different ages in the same infant.

**Aims:** Our aim was to gain a more complete knowledge of the postnatal maturation and consistency of the initial phase of the HVR in human term infants in both quiet and active sleep (AS), by performing multiple tests during the first 6 months of life. We hypothesised that sleep state would have a marked effect on the HVR and that a significant maturation would be observed.

**Methods:** Fifteen healthy term infants, born at 38-41 weeks' gestational age, were studied at 2-5 weeks, 2-3 and 5-6 months after birth. Daytime polysomnography was performed between 10:00 and 16:00 h. Nasal airflow was measured using a miniaturised pneumotachograph attached to a silicone nose-mask (Parslow et al, *Respir Physiol Neurobiol* 136: 235-247, 2003). Each infant was presented with a mildly hypoxic gas mixture (15% O<sub>2</sub>, balance N<sub>2</sub>) at least three times during both AS and QS. Tests were terminated if: the infant aroused, after 5 minutes with no arousal, or when SpO<sub>2</sub> fell below 85%.

**Data Analysis:** Mean values of oxygen saturation (SpO<sub>2</sub>) and inspired minute ventilation ( $V'_I$  (mL/min/kg)) were calculated for the initial 15s and subsequent 30s epochs of the hypoxic test period. Ventilatory responses were expressed as percentage changes relative to baseline values obtained in the one minute preceding each hypoxic test. Data from tests repeated within infants were averaged for each sleep state; arousing and non arousing tests were analysed separately. Standard deviations between infants were compared using F-tests to investigate the effects of sleep state and age.

**Results:** At each age, mild hypoxia induced a significant decrease in SpO<sub>2</sub> during both sleep states, regardless of whether or not the infant aroused. In AS infants consistently aroused; however, in QS infants both aroused and failed to arouse to tests. The initial HVR varied considerably between infants;  $V'_I$  increased, decreased, or was unchanged. The response was markedly more variable during AS compared with QS at 2-5 weeks (15s; 30s,  $p < 0.05$ ) and 2-3 months (30s,  $p < 0.05$ ). There was no significant difference between sleep states at 5-6 months.

**Conclusions:** This study has demonstrated that in healthy term infants the initial phase of the HVR exhibits considerable variability between infants, with responses being markedly more variable during AS than QS. With increasing postnatal age the responses become more consistent between tests. By performing single hypoxic tests and by failing to account for arousal responses, previous studies may have underestimated the natural variation of the HVR.

**IMAGING LUNG AERATION AFTER BIRTH USING A SYNCHROTRON.**

**S.B. Hooper**<sup>1</sup>, M.J. Wallace<sup>1</sup>, M. Morgan<sup>2</sup>, I. Williams<sup>2</sup>, C. Hall, K. Sui<sup>2</sup> & R. Lewis<sup>2</sup>  
Department of Physiology<sup>1</sup> and School of Physics and Materials Engineering<sup>2</sup>, Monash  
University, 3800, Victoria, Australia. E-mail: stuart.hooper@med.monash.edu.au

The survival of newborn infants at birth is critically dependent upon the lungs successfully taking over the role of respiratory gas exchange. Before birth, gas exchange occurs across the placenta and the lungs are filled with liquid. Thus, at birth the lungs must be cleared of liquid to allow the entry of air, yet a thin film must remain to protect the epithelial cells lining the airway from desiccation. However, the factors that regulate and influence lung aeration at birth are unknown because, until recently, no technique has been able to measure it. Instead, most studies have focussed on measuring liquid clearance from the airways, but these studies have provided no information on the rate and pattern of lung aeration at birth or the factors that affect it. Even simple questions remain unanswered. For example, what is the effect of gravity and, therefore, body position on the pattern of lung aeration? It is often assumed that airway liquid will gravitate towards the lower regions of the lungs depending upon body position, thereby reducing or preventing gas exchange in these regions. Our aim was to utilize the recently developed method of phase contrast X-ray imaging to study the rate and pattern of lung aeration at birth and the factors that may affect it.

Conventional absorption x-ray imaging is the most commonly used biomedical imaging technique, but has little ability to resolve soft tissues such as the lung. Although other imaging modalities, such as MRI, can produce high contrast images of soft tissue, movement artefacts are a significant problem with lung imaging, requiring short exposure times. We have utilized the newly developed technique of phase contrast X-ray imaging, to image the rate and pattern of lung aeration after birth. Phase contrast X-ray imaging utilizes differences in the refractive index between air and water to reveal the airways with striking clarity. After birth, the lung is predominantly comprised of air (~80% by volume) which contrasts strongly with the surrounding tissue structures that are comprised mainly of water. This feature allows the air-filled lung to be imaged with remarkably high phase contrast (100 times better than absorption contrast) and spatial resolution (better than 100 micron). We used a synchrotron (SPring-8, Japan) as the radiation source, because its brightness and high beam intensity allows the use of monochromatic radiation and short exposure times, thereby greatly improving image resolution.

At 29-31 days of gestation (term is ~32 days) pregnant rabbits (n=9) were anaesthetized, pups were delivered by caesarian section and either imaged live from birth (n=5) or were killed at selected periods after birth before they were imaged. Images of live pups were collected at 10 sec intervals for the first hour after birth. Pups imaged after death were killed at <5mins, 10 mins, 15mins, 30mins, 1 hour and 2 hours after the onset of ventilation (n=4 to 5 per time point); pups killed before the onset of ventilation were also imaged.

Our results demonstrate that the ability of phase contrast X-ray imaging to clearly resolve the airways is due to the presence of air as the liquid-filled airways cannot be resolved using this technique; the resolution was remarkably good and is able to resolve airways of <100µm in diameter. Lung aeration at birth is gradual, beginning within the central, large airways and gradually progressing to the peripheral regions. The basal regions of both lobes are the last to aerate and there is a marked effect of body position, with the lower regions aerating at significantly reduced rates.

Our study clearly demonstrates that phase contrast X-ray imaging is ideal for imaging the airways of the lung. As a result we have, for the first time, provided information on the rate and pattern of lung aeration after birth. We are currently developing mathematical equations that will allow the calculation of air volumes and the size distribution of respiratory units which will greatly advance the diagnosis of lung disease states such as COPD.

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# Registrants

**Ms. Beth Allison**  
Monash University  
Melbourne  
beth.allison@med.monash.edu.au

**Dr. Jane Black**  
Monash University  
Melbourne  
jane.black@med.monash.edu.au

**Dr. Frank Bloomfield**  
Liggins Institute  
Auckland, NZ  
f.bloomfield@auckland.ac.nz

**Dr. Wee-Ming Boon**  
Monash University  
Melbourne  
wee.boon@med.monash.edu.au

**Ms. Kimberley Botting**  
Adelaide  
kimberley.botting@student.adelaide.edu.au

**Mr. John Bromfield**  
University of Adelaide  
Adelaide  
john.bromfield@adelaide.edu.au

**Ms. Kristen Bubb**  
Monash University  
Melbourne  
kristen.bubb@med.monash.edu.au

**Mr. Bernard Chuang**  
Royal Adelaide Hospital  
Adelaide  
hui.chuang@student.adelaide.edu.au

**Dr. Vicki Clifton**  
John Hunter Hospital  
Newcastle  
Vicki.Clifton@newcastle.edu.au

**Dr. Heather Coughtry**  
WAHS  
Sydney  
coughth@wahs.nsw.gov.au

**Ms. Penny Dalitz**  
Monash University  
Melbourne  
penelope.dalitz@med.monash.edu.au

**Dr. Miles De Blasio**  
University of Adelaide  
Adelaide  
miles.deblasio@adelaide.edu.au

**Dr. Kate Denton**  
Monash University  
Melbourne  
kate.denton@med.monash.edu.au

**Ms. Hayley Dickinson**  
Monash University  
Melbourne  
hayley.dickinson@med.monash.edu.au

**Ms. Jamie Duffield**  
University of Adelaide  
Adelaide  
jaime.duffield@adelaide.edu.au

**Ms. Jessica Faggian**  
Monash University  
Melbourne  
jessica.faggian@med.monash.edu.au

**Ms. Kirsten Ferrand**  
University of Adelaide  
Adelaide  
kirsten.farrand@adelaide.edu.au

**Dr. Susan Feng**  
Monash Medical Centre  
Melbourne  
ying.feng@med.monash.edu.au

**Ms. Caitlin Filby**  
Monash University  
Melbourne  
caitlin.filby@med.monash.edu.au

**Dr. Sharon Flecknoe**  
Monash University  
Melbourne  
sharon.flecknoe@med.monash.edu.au

**Mrs. Rebecca Flower**  
Monash University  
Melbourne  
Rebecca.flower@med.monash.edu.au

**Dr. Kathy Gatford**  
University of Adelaide  
Adelaide  
kathy.gatford@adelaide.edu.au

**Ms. Sheridan Gentili**  
University of Adelaide  
Adelaide  
sheridan.gentili@adelaide.edu.au

**Dr. Karen Gibson**  
University of NSW  
Sydney  
k.gibson@unsw.edu.au

**Prof. Jane Harding**  
Liggins Institute  
Auckland, NZ  
j.harding@auckland.ac.nz

**Prof. Richard Harding**  
Monash University  
Melbourne  
richard.harding@med.monash.edu.au

**Mrs. Cherise Hillier Fletcher**  
University of Adelaide  
Adelaide  
cherise.hillier@adelaide.edu.au

**Dr. Jon Hirst**  
Monash University  
Melbourne  
jon.hirst@med.monash.edu.au

**Dr. Deborah Hodgson**  
University of Newcastle  
Newcastle  
deborah.hodgson@newcastle.edu.au

**A. Prof. Stuart Hooper**  
Monash University  
Melbourne  
stuart.hooper@med.monash.edu.au

**Ms. Chantal Hoppe**  
Monash University  
Melbourne  
chantal.hoppe@med.monash.edu.au

**Dr. Rosemary Horne**  
Monash Medical Centre  
Melbourne  
rosemary.horne@med.monash.edu.au

**Dr. Anne Jaquiere**  
Liggins Institute  
Auckland, NZ  
a.jaquiere@auckland.ac.nz

**Dr. Catriona Jenkinson**  
Massey University  
Palmerston North, NZ  
c.m.jenkinson@massey.ac.nz

**Dr. Zsuzsoka Kecskes**  
The Canberra Hospital  
Woden, ACT  
zsuzsoka.kecsek@act.gov.au

**Dr. Alison Kent**  
The Canberra Hospital  
Woden, ACT  
alison.kent@act.gov.au

**Dr. Michelle Kett**  
Monash University  
Melbourne  
michelle.kett@med.monash.edu.au

**Dr. Paul Kenyon**  
Massey University  
Palmerston North, NZ  
p.r.kenyon@massey.ac.nz

**Dr. Karen Kind**  
University of Adelaide  
Adelaide  
karen.kind@adelaide.edu.au

**Dr. Leo Leader**  
University of NSW  
Sydney  
l.leader@unsw.edu.au

**Ms. Lucy Liu**  
University of Adelaide  
Adelaide  
qian.liu@student.adelaide.edu.au

**Prof. Eugenie Lumbers**  
University of NSW  
Sydney  
e.lumbers@unsw.edu.au

**Mr. Severence MacLaughlin**

University of Adelaide  
Adelaide  
Smm27@cornell.edu

**Mr. Albert Matti**

University of Adelaide  
Adelaide  
albert.matti@student.adelaide.edu.au

**Prof. Caroline McMillen**

University of Adelaide  
Adelaide  
caroline.mcmillen@adelaide.edu.au

**Dr. Karen Moritz**

Monash University  
Melbourne  
karen.moritz@med.monash.edu.au

**Dr. Janna Morrison**

University of Adelaide  
Adelaide  
janna.morrison@adelaide.edu.au

**Ms. Beverly Muhlhausler**

University of Adelaide  
Adelaide  
Beverly.muhlhausler@adelaide.edu.au

**Dr. Makoto Nakamura**

Monash Medical Centre  
Melbourne  
JCB01257@nifty.com

**Ms. Rachael Nowack**

University of Adelaide  
Adelaide  
rachael.nowak@imvs.sa.gov.au

**Ms. Amanda O'Connell**

University of NSW  
Sydney  
a.oconnell@student.unsw.edu.au

**Prof. Julie Owens**

Adelaide University  
Adelaide  
julie.owens@adelaide.edu.au

**Dr. Nayana Anupam Parange**

University of Adelaide  
Adelaide  
nanya.parange@adelaide.edu.au

**Dr. Jane Pillow**

Memorial Hospital  
Perth  
janep@ichr.uwo.edu.au

**Ms. Kirsty Pringle**

Adelaide University  
Adelaide  
kirsty.pringle@adelaide.edu.au

**Dr. Megan Probyn**

Monash University  
Melbourne  
megan.probyn@med.monash.edu.au

**Ms. Leewen Rattanatr**

University of Adelaide  
Adelaide  
leewen.rattanatr@student.adelaide.edu.au

**Ms. Heidi Richardson**

Monash Medical Centre  
Melbourne  
heidi.richardson@med.monash.edu.au

**Mr. Vincent Ritorto**

University of Adelaide  
Adelaide

**Dr. Claire Roberts**

University of Adelaide  
Adelaide  
claire.roberts@adelaide.edu.au

**Dr. Christopher Rumball**

University of Auckland  
Auckland  
c.rumball@auckland.ac.nz

**Dr. Jeff Schwartz**

University of Adelaide  
Adelaide  
jeff.schwartz@Adelaide.edu.au

**Ms. Amanda Sferruzzi-Perri**

Adelaide University  
Adelaide  
Amanda.sferruzzi-perri@adelaide.edu.au

**Ms. Reetu Singh**

Monash University  
Melbourne  
reetu.singh@med.monash.edu.au

**Mr. Andrew Snell**  
University of Adelaide  
Adelaide  
Andrew.snell@adelaide.edu.au

**Ms. Foula Sozo**  
Monash University  
Melbourne  
foula.sozo@med.monash.edu.au

**Mr. Timothy Spencer**  
University of Adelaide  
Adelaide  
timothy.spencer@adelaide.edu.au

**Ms. Prue Standen**  
University of Adelaide  
Adelaide  
prue.standen@student.adelaide.edu.au

**Dr. Michael Stark**  
John Hunter Hospital  
Newcastle  
Michael.J.Stark@studentmail.newcastle.edu.au

**Dr. Keiji Suzuki**  
Monash University  
Melbourne  
dks@med.monash.edu.au

**Dr. Marianne Tare**  
Monash University  
Melbourne  
Marianne.tare@med.monash.edu

**Ms. Alison Thiel**  
Monash University  
Melbourne  
alison.thiel@med.monash.edu

**Mr. Eric Thorstensen**  
University of Auckland  
Auckland  
e.thorstensen@auckland.ac.nz

**Dr. David Todd**  
Westmead Hospital  
Westmead  
david\_todd@wsahs.nsw.gov.au

**Mr. Andrew Trotta**  
University of Adelaide  
Adelaide  
Andrew.trotta@student.adelaide.edu.au

**Dr. Rita Tuladhar**  
Mercy Hospital  
Melbourne  
rita\_tuladhar@hotmail.com

**Prof. Adrian Walker**  
Monash Medical Centre  
Melbourne  
Adrian.walker@med.monash.edu.au

**Dr. Megan Wallace**  
Monash University  
Melbourne  
megan.wallace@med.monash.edu.au

**Prof. Marelyn Wintour**  
Monash University  
Melbourne  
mwc@med.monash.edu.au

**Dr. Ian Wright**  
University of Newcastle  
Newcastle, NSW  
iwright@mail.newcastle.edu.au

**Ms. Olivia Wyss**  
University of Adelaide  
Adelaide  
olivia.wyss@adelaide.edu.au

**Ms. Stephanie Yiallourou**  
Monash Medical Centre  
Melbourne  
Stephanie.yiallourou@med.monash.edu.au

**Ms. Valerie Zahra**  
Monash University  
Melbourne  
Valerie.zahra@med.monash.edu.au

**Ms. Gosia Zieba**  
Monash University  
Melbourne  
Malgorzata.Zieba@med.monash.edu.au