# 28th Annual Meeting of The Fetal and Neonatal Workshop of Australia and New Zealand

Yanchep Inn
Yanchep, Western Australia
4-5 April, 2014


2014 Organising Committee

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|---|---|
| Jane Pillow | University of Western Australia |
| Rob De Matteo | Monash University |
| Richard Harding | Monash University |

Affiliated with the Perinatal Society of Australia and New Zealand Ltd

# Program Outline

**CONFERENCE VENUE**

Yanchep Inn
Yanchep National Park
Yanchep, Western Australia

| FRIDAY 4TH APRIL | |
|---|---|
| ***9.30am-10.30am*** | **Registration** |
| ***10.30am-11.45am*** | **Session 1** |
| ***11.45am-12.15pm*** | **Morning Tea** |
| ***12.15pm-1.30pm*** | **Session 2** |
| ***1.30pm-2.30pm*** | **Lunch** |
| ***2.30pm-3.45pm*** | **Session 3** |
| ***3.45pm-4.15pm*** | **Afternoon Tea** |
| ***4.15pm-5.30pm*** | **Session 4** |
| ***6.30pm*** | **Conference Dinner Cabaret Cave** |

| SATURDAY 5TH APRIL | |
|---|---|
| ***9.30am-10.00am*** | **Registration** |
| ***10.00am-11.30am*** | **Session 5** |
| ***11.30am-12.00pm*** | **Morning Tea** |
| ***12.00pm-1.30pm*** | **Session 6** |
| ***1.30pm-2.30pm*** | **Lunch** |
| ***2.30pm-3.45pm*** | **Session 7** |
| ***3.45pm-4.15pm*** | **Afternoon Tea** |
| ***4.15pm-5.15pm*** | **Session 8** |
| ***5.20pm*** | **Presentation of student prizes** |
| ***5.30pm*** | **Workshop 2015** |
| ***5.30pm*** | **Close of Workshop 2014** |
| ***6.00pm*** | **Bus departs for Perth** |

# Scientific Program

## DAY 1- FRIDAY 4th APRIL

*Registration: 9.30am-10.30am*

*E=Early PhD, L=Late PhD*

| Session 1: Chairs – Sarah Robertson and Ian Wright | | | |
|---|---|---|---|
| 10.30am | **A1** | Claire Roberts | Human studies of pregnancy outcome: trials and tribulations |
| 10.45am | **A2** | Jeffrey Craig | Can we do more to help parents of newborn twins understand about zygosity and chorionicity? |
| 11.00am | **A3** | Angela Cumberland (E) | Long term consequences of the suppression of neurosteroid production in late gestation |
| 11.15am | **A4** | Damien Hunter (E) | Low birth-weight and poor postnatal growth correlate with poorer memory and cognitive flexibility in male IUGR sheep in maze tasks |
| 11.30am | **General discussion** | | |

*Morning tea: 11.45am-12.15pm*

| Session 2: Chairs – Kathy Gatford and Jon Hirst | | | |
|---|---|---|---|
| 12.15pm | **A5** | Kai Yie Tay (L) | Effect of preterm birth on cardiac structure and vessels in lambs |
| 12.30pm | **A6** | Vivian Nguyen (L) | The effect of moderate preterm birth on the hearts of lambs 2 day after birth |
| 12.45pm | **A7** | Maria Nguyen (L) | Does intrauterine inflammation affect atherosclerotic lesion development and endothelial function in ApoE-/- mice? |
| 1.00pm | **A8** | Barbara Lingwood | Angiotensin levels in preterm babies |
| 1.15pm | **General discussion** | | |

*Lunch: 1.30pm-2.30pm*

| Session 3: Chairs – Megan Wallace and Barbara Lingwood | | | |
|---|---|---|---|
| 2.30pm | **A9** | Yong Song | Impact of mechanical ventilation on preterm diaphragm and protective effect of Vitamin A and Retinoic Acid |
| 2.45pm | **A10** | Takushi Hanita | Effects of severe intrauterine inflammation on mechanically ventilated preterm lambs |
| 3.00pm | **A11** | Justin Lang (L) | Partial aeration with 100% oxygen at birth increases pulmonary blood flow in aerated and non-aerated regions |
| 3.15pm | **A12** | Sheena Bouch (L) | Does an impaired antioxidant system modify the effects of neonatal hyperoxia on lung development? |
| 3.30pm | **General discussion** | | |

*Afternoon tea: 3.45pm-4.15pm*

| Session 4: Chairs – Graham Jenkin and Vicki Clifton | | | |
|---|---|---|---|
| 4.15pm | **A13** | Sarah Robertson | Macrophages prevent spontaneous preterm birth in mice |
| 4.30pm | **A14** | Natalie Aboustate (E) | Expression of MicroRNAs that regulate inflammation in cord blood from preterm neonates |
| 4.45pm | **A15** | Tim Moss | Influence of human amnion epithelial cells on the fetal inflammatory response |
| 5.00pm | **A16** | Amy Wooldridge (E) | Effect of placental restriction and late maternal methyl supplementation on immune functional outcomes in sheep |
| 5.15pm | **General discussion** | | |

***Conference dinner: Cabaret Caves, 1830pm***

## DAY 2- SATURDAY 5th APRIL

*Registration: 9.30am-10.00am*

*E=Early PhD, L=Late PhD, H=Honours*

| Session 5: Chairs – Leo Leader and Julie Pitcher | | | |
|---|---|---|---|
| 10.00am | **A17** | Julia Shaw (E) | Characterization of GABAA receptors in the preterm brain |
| 10.15am | **A18** | Lorna McKerracher | What effect do antenatal steroids and magnesium sulphate have on neurotrophic factors in preterm infants? |
| 10.30am | **A19** | Robert Galinsky | Magnesium for perinatal hypoxia-ischemia in term infants: A systematic review of preclinical studies |
| 10.45am | **A20** | Courtney McDonald | Neuroprotective effects of hAECs following neonatal hyperoxia |
| 11.00am | **A21** | Samantha Barton (L) | Localisation of human amnion epithelial cells to the cerebral white matter is not required for neuroprotection |
| 11.15am | **General discussion** | | |

*Morning tea: 11.30am-12.00pm*

| Session 6: Chairs – Jane Pillow and Michael Stark | | | |
|---|---|---|---|
| 12.00pm | **A22** | Kurt Albertine | Epigenetic basis of lung and brain damage in chronically ventilated preterm lambs |
| 12.30pm | **A23** | Sonia Sam (L) | Adaptation of the cardiac renin-angiotensin system (RAS) to extrauterine life in term and preterm piglets |
| 12.45pm | **A24** | Kathryn Gatford | Premature birth and metabolic outcomes in sheep |
| 1.00pm | **A25** | Melinda Dolan (L) | The role of fibroblast growth factors in inflammation-induced fetal lung maturation |
| 1.15pm | **General discussion** | | |

*Lunch: 1.30pm-2.30pm*

| Session 7: Chairs – Julie Owens and Tim Moss | | | |
|---|---|---|---|
| 2.30pm | **A26** | Yu Wang | Regulation of renin angiotensin system (RAS) pathways in the human decidua |
| 2.45pm | **A27** | Jessica Laurence (L) | Vitamin D and IGF gene expression in first trimester and term placentae |
| 3.00pm | **A28** | Vicki Clifton | Placental GR isoform expression varies in relation to gestational age at delivery in a sex specific manner |
| 3.15pm | **A29** | Hong Liu (L) | Can neonatal exendin-4 prevent diabetes after IUGR? |
| 3.30pm | **General discussion** | | |

*Afternoon tea: 3.45pm-4.15pm*

| Session 8: Chairs – Alison Kent and Claire Roberts | | | |
|---|---|---|---|
| 4.15pm | **A30** | Dana Ryan (L) | Does IUGR have an effect on nephrogenesis in the developing human kidney? |
| 4.30pm | **A31** | Kirsty Pringle | Preliminary observations on the effect of maternal health on fetal body and kidney growth in Indigenous women |
| 4.45pm | **A32** | Stacey Ellery (L) | Permanent nephron loss following neonatal acute kidney injury in male offspring, does this lead to chronic kidney disease? |
| 5.00pm | **General discussion** | | |
| | | | |
| 5.25pm | **Presentation of Student Prizes** | | |
| 5.30pm | **Fetal and Neonatal Workshop 2015** | | |
| 5.30pm | **Close of Workshop** | | |

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

### Terms of reference

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

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

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# Human studies of pregnancy outcome: trials and tribulations

Claire Roberts[1], Shalem Leemaqz[1], Ang Zhou1, Lesley McCowan[2], Gus Dekker[1]

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**Background:** Pregnancy complications, including IUGR, preterm birth (PTB), preeclampsia (PE) and gestational diabetes (GDM), together afflict about 25% of pregnancies. They can be life threatening to mother or infant in about 6% of pregnancies. However, in nulliparous women we have no way of knowing which women will develop these conditions. We do know that the placenta is central to pregnancy success.

**Aims/Hypothesis:** We aimed to develop screening tools to identify women at risk in early pregnancy before symptoms manifest. We hypothesised that genetic, clinical and lifestyle factors can be used to predict risk in first pregnancies.

**Methods:** We used logistic regression analyses of clinical, lifestyle, dietary, socioeconomic, family history and genotype data for Caucasian mother, father, baby, trios in the Adelaide & Auckland SCOPE cohorts (n=3229) to identify couples at risk.

**Results:** Population differences make the task of identifying risk factors more difficult than expected. Socioeconomic status, family history, diet, supplement use, smoking, illicit drug use, physical activity, maternal and paternal BMI, and other factors are different between the Adelaide and Auckland SCOPE cohorts. The Adelaide cohort was a comparatively disadvantaged population with low socioeconomic status who suffered 2-4 times the rate of pregnancy complications compared to the Auckland SCOPE women. Maternal consumption of fruit and vegetables before and during pregnancy, which reduce risk for adverse pregnancy outcomes, was significantly more frequent in Auckland women. Continued cigarette smoking and marijuana use was 10 times more common in Adelaide than Auckland women. As for most complex diseases, gene variants that associate with pregnancy complications have low penetrance but interact with modifiable and non-modifiable risk factors including fetal sex to influence maternal and infant health. Some of these factors are associated with more than one pregnancy complication, likely through actions in the placenta.

**Conclusions:** The development of really effective screening tools to predict risk in first pregnancies across populations requires collection of detailed data from large numbers of women. Despite the difficulties it is an important goal in pregnancy research because of the health consequences for mother and child both in the short and long term.

# Can we do more to help parents of newborn twins understand about zygosity and chorionicity?

Tessa Cutler[1], Louise Keogh[2], Kate Murphy[1] John Hopper[1] & Jeffrey M Craig[3]

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**Background:** For many years, twin research has provided insights into the genetic and environmental origins of health and disease. Such studies have relied on estimates of zygosity based on sex and chorionicity. However, the extent to which twins and parents of twins are informed about and understand zygosity is unknown.

**Aims/Hypothesis:** Our aims were twofold: First, we aimed to ascertain the levels of zygosity knowledge in adult twins and parents of twin children and what this knowledge means to them. Second, armed with this data and educational tools that we have developed, we aimed to increase knowledge amongst stakeholder groups.

**Methods:** At the 2012 TwinsPlus Festival we offered free zygosity testing for twins and parents of twins who were at all unsure about their zygosity status. Buccal DNA was sent to the Australian Genome Research Centre for a 12-marker zygosity test and results were fed back to participants.

**Results:** Of 125 pairs from which a cheek swab was provided, more than 90% were found from DNA testing to be monozygotic. One in five of the tested twins had incorrectly thought they were dizygotic. Four-fifths said that they had received misinformation from medical professionals. Incorrect calls were based on the false assumptions that two placentas meant dizygotic twins and/or that "identical" twins always look identical. Parents and adult twin pairs alike said that it was very important for them to know the true zygosity and many were extremely surprised at the results.

**Conclusions:** We conclude that zygosity knowledge amongst twins and parents of twins needs to be improved. We recommend that all parents of newborn twins in Australia should be provided with written information on the twins' chorionicity and zygosity if possible and if same-sex, parents should be provided with details of discounted zygosity testing via the Australian Twin Registry.

# Long Term Consequences of the Suppression of Neurosteroid Production in Late Gestation

Angela L Cumberland[1], Hannah K Palliser[1], David W Walker[2] & Jonathan J Hirst[1]

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**Background:** Neuroactive steroids have a major role in brain development and are particularly important in the maturation of oligodendrocytes and myelination. Studies investigating the inhibition of the production of key neurosteroid allopregnanolone, using the 5$\alpha$-reductase inhibitor finasteride, have shown marked reductions in mature myelin, as well as increased apoptosis within finasteride-treated fetal brains. However, little is known about the long-term consequences of a low neurosteroid environment during pregnancy.

**Aims/Hypothesis:** The aim of this study was to investigate the alterations in behaviour and myelination in neonatal guinea pigs, following inhibition of neurosteroid production during pregnancy.

**Methods:** Time-mated, outbred pregnant guinea pig dams received oral administration of vehicle (45% $\beta$-cyclodextrin) or finasteride (25mg/kg) once daily, commencing at GA60 until delivery (term ~71 days). At postnatal day (PND) 7 all pups underwent behavioural testing using open field (OF) and novel object recognition tests (NORT). Neonates were also scored on 4 behavioural outcomes – vocalisations, hiding, escape and stationary behaviours. At post mortem on PND8, brains were collected and analysed for area coverage of MBP in lobes VIII and X, and deep white matter of the cerebellum.

**Results:** Female neonates exposed to finasteride spent less time exploring the inner zone compared to control females ($p=0.0047$). NORT showed no significant differences. However, finasteride-exposed animals appeared to spend less time exploring either the familiar or novel object in NORT1 and 2 ($p=0.091$ and $p=0.12$ respectively). This difference appeared to be greater in finasteride treated females. No differences were found in myelination (MBP area coverage) between sex or neurosteroid environment in pregnancy.

**Conclusion:** These observations suggest there is "catch-up" development of the mature myelinating cells (MBP) from offspring exposed to finasteride *in utero.* However, a reduced neurosteroid environment has long-term effects on offspring behaviour, with a greatest affect on females. This change suggests female neonates develop a more anxious phenotype following neurosteroid suppression during pregnancy than males. These results further imply that, whilst myelin coverage recovers, there is still potential damage in programming of behaviours.

# Low birth-weight and poor postnatal growth correlate with poorer memory and cognitive flexibility in male IUGR sheep in maze tasks

Damien S. Hunter[1,2,3,] Susan J. Hazel[3], Karen L. Kind[1,3,] Hong Liu[1,2], Danila Marini[3], Lynne Giles[1,4], Julie A. Owens[1,2,] Julia B. Pitcher[1,2], Kathryn L. Gatford[1,2]

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**Background:** Catch-up growth in the first six months of life ameliorates the adverse effect of intrauterine growth restriction (IUGR) on learning, memory and cognitive flexibility in humans[1]. Assessing the relative contributions of prenatal and postnatal growth is difficult in humans due to a range of confounding factors that can affect neurodevelopment. We therefore examined the relationship of birth weight and neonatal growth with measures of learning, memory and cognitive function in adolescence, in control sheep and in sheep whose growth was restricted before birth.

**Hypothesis:** Birth weight and neonatal growth rate will correlate negatively with number of trials and time required to solve maze learning tasks in adolescent sheep.

**Methods:** Low birth weight was induced by surgical reduction of placental implantation sites (PR) and by natural twinning. Birth weight (BW), and fractional growth rate (FGR) during the first 16 days of life (during rapid neonatal catch-up growth in this species) were measured in control (23M, 17F) and PR (6M, 10F) sheep. Trials and time per task were recorded for initial learning (L), memory (M1, M2) and reversal (R1, R2) maze tasks at 18 and 40 weeks of age[2,3]. Relationships between BW and FGR and outcomes were analysed using multiple linear regression modelling (time per task) or Poisson regression modelling (numbers of trials per task), with significance for effects of BW or FGR accepted at $p<0.05$.

**Results:** When both sexes were examined together, BW and FGR did not affect learning at 18 weeks. Also in both sexes, time to solve Task M1 at 40 weeks correlated negatively with FGR (std $\beta = -0.29$, $p=0.026$) and tended to correlate negatively with BW (std $\beta = -0.24$, $p=0.074$). Other correlations at 40 weeks were sex specific: number of trials to solve Task M1 correlated negatively with FGR (std $\beta = -0.23$, $p=0.025$), and also tended to with BW (std $\beta = -0.24$, $p=0.09$) in males, but not females (both $p>0.8$). Number of trials to solve Task M2 tended to correlate negatively with FGR in males (std $\beta = -0.20$, $p=0.098$) but not females ($p>0.3$).

**Conclusions:** As with humans, slow neonatal growth rate in sheep correlated with impaired learning performance on a memory task. This was most evident in males, and after puberty. Effects of variation in BW were limited, with some indication of negative effects of low BW in males, but not females. Interventions to promote neonatal growth may be beneficial for cognitive outcomes, particularly for males; the sheep may provide a useful model for such investigations.

[1.] Fattal-Valevski et al. (2009) *J Child Neurol*, 24 (7). [2.] Hernandez et al., (2009) Behav Brain Res, 204(1),
[3.] Erhard et al., (2004) Behav Brain Res, 151 (1-2)

# NOTES

# Effect of preterm birth on cardiac structure and vessels in lambs

Kai Yie Tay[1], Ian LeGrice[2, 3], Gregory Sands[2,3], Frank Bloomfield [1, 4, 5], Jane Harding[1] Mark Oliver[1,4], Anne Jaquiery[1,4,5]

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**Background:** Preterm birth is associated with increased risk of cardiovascular disease in later life. Preterm infants are exposed to a different environment (extrauterine instead of intrauterine) in early life and this might induce abnormal development of the structure of the heart, an organ undergoing significant changes around birth. Studying the development of myocardial and coronary vascular architecture after preterm birth is important for understanding the origins of increased risk of cardiovascular disease.

**Aims/Hypothesis:** To determine the effects of preterm birth on postnatal myocardial organisation and cardiac vascular architecture. / Preterm birth alters myocardial organisation and coronary vasculature affecting later cardiac function

**Methods:** Twin-bearing ewes were randomly assigned to induced premature delivery at 137 days' gestation, dGA (term = 147 dGA) or to deliver spontaneously. To investigate cardiac development, hearts were excised on day 2 or 11 after birth and were perfusion-stained with picrosirius red dye and fixed in Bouin's solution. Transmural blocks were cut from left and right ventricular free walls and interventricular septum. Tissue blocks were embedded in resin and prepared for extended volume confocal microscopy for assessing myocardial architecture. In a second series of hearts, a silicone elastomer-based X-ray contrast agent (Microfil®) was injected into the coronary arteries. The heart was then imaged in a micro-CT scanner and the coronary arterial tree was extracted from the 3D CT images for quantitative analysis.

**Results:** To date, 73 hearts have been collected. The tissue preparation, imaging and image analysis protocols have been developed and trialled.

**Conclusions:** We have successfully optimized the protocol for the studies to be conducted. Cardiac imaging will allow quantification of alterations in myocardial organisation and coronary vasculature after preterm birth, providing novel insights into the structural and functional effects of preterm birth on the developing heart.

# The effect of moderate preterm birth on the hearts of lambs 2 day after birth

Vivian Nguyen[1], Robert De Matteo[1], Richard Harding[1], *Graeme Polglase[2] & *M Jane Black[1]

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**Background:** A major haemodynamic transition occurs at birth whereby arterial pressure and heart rate rise dramatically. Preterm birth (<37 weeks) accounts for ~8% of all live births in Australia, with the majority (80%) born moderately preterm. It is therefore important to understand how the heart adapts to preterm birth, in particular the growth and maturity of cardiomyocytes, compared to term infants.

**Aim:** This study aims to compare cardiac structure and cardiomyocyte number in the ventricles of moderately preterm and term lambs in the immediate period after birth.

Methods: Pregnant ewes carrying singleton fetuses were induced (by Epostane) to deliver vaginally at 0.9 of term (132±1 days of gestation; moderately preterm; n=13) or at term (≈147 days, n=15). To facilitate survival of preterm lambs, clinically relevant doses of Betamethasone were administered 24h and 48h before birth. At 2 days after birth, hearts were excised at necropsy, weighed and perfusion fixed. The ventricles were sectioned transversely (10mm slices) and ventricular wall thickness determined using image analysis. Ventricular wall and chamber volumes were estimated using the Cavalieri principle. Using a smooth fractionator approach, the left ventricle plus septum (LV+S) and right ventricle (RV) were sampled, embedded in glycomethacrylate and the number of cardiomyocyte nuclei determined.

**Results:** Preterm lambs were significantly lighter at birth and at necropsy compared to term lambs. At 2 days after birth, preterm lambs had thinner RV (P=0.002), LV (P=0.001) and septum (P=0.03) walls; however when adjusted for body weight, RV (P<0.0001), LV (P<0.0001) and septum (P<0.0001) thicknesses were greater. Likewise, ventricular wall volume of the RV (P<0.0001) and LV+S (P<0.0001) was less in preterm hearts but there were no differences relative to body weight. Preterm lambs exhibited smaller RV (P<0.0001) and LV (P=0.002) chamber volumes compared to term lambs, but when adjusted for body weight only RV chamber volume remained significantly smaller (P=0.029).

**Conclusions:** As expected at 2 days after birth, absolute size of the ventricles was reduced in the preterm hearts compared to controls. Since the proliferative capacity of cardiomyocytes is markedly reduced postnatally, it is imperative to determine if this affects the number of cardiomyocytes in the preterm heart as this has the potential to impact on lifelong functional reserve. This component of the study is still ongoing.

# Does intrauterine inflammation affect atherosclerotic lesion development and endothelial function in *ApoE*$^{-/-}$ mice?

Maria Nguyen[1,2], Megan Wallace[1,2], Trevelyan Menheniott[3], Salvatore Pepe[3,4], Tracey Gaspari[5], Robert Widdop[5], David Burgner[3,4]*, Timothy Moss[1,2]*

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**Background:** Atherosclerosis is a chronic condition that may initiate prenataly, particularly under the influence of inflammatory pathways. The impact of intrauterine inflammation (chorioamnionitis) on the development of early atherosclerosis is suspected but has not been demonstrated. Here, we report preliminary findings from a novel mouse model of chorioamnionitis and atherosclerosis.

**Aims/Hypothesis:** We investigated the effect of chorioamnionitis on the development of early atherosclerosis in mice.

**Methods:** Apolipoprotein E deficient (*ApoE*$^{-/-}$) pregnant mice (n=15-19 per group) received LPS (0.1 ng in 5 µl saline) or saline (5 µl) into each amniotic sac on gestational day 15. Offspring were weaned onto a high fat (21% v/v) diet at 4 weeks. Aortae, heart, and plasma were collected from 6,12- and 20-week-old offspring. Aortae were analysed histologically following Haematoxylin and Eosin (H&E) as well as Oil Red O staining. Heart and aortae cholesterol concentrations were quantified by colourimetry. Endothelial vasodilation in response to acetylcholine was tested in abdominal aortae of 20-week-old offspring.

**Results:** Intra-amniotic LPS injection induced histological chorioamnionitis. 6-week-old LPS-treated mice developed increased heart and aortae cholesterol concentrations ($p<0.05$). Lesion development, as assessed by aortic sinus fatty deposits (Oil Red O) and intima to media ratio, and an *en face* analysis of Oil Red O-stained aorta, did not differ between LPS- and saline-treated animals at 6 or 12 weeks. In 12-week-old mice, reduced mean intima to media ratio ($p=0.02$) was found in brachiocephalic artery sections from LPS-treated offspring. Sex differences in lesion development exist in 6- and 12-week old, LPS-treated *ApoE*$^{-/-}$ mice. In 20-week-old mice, endothelium-dependent vasodilation did not differ between sex or treatment group.

**Conclusions:** Contrary to expectation, 12-week-old LPS-treated mice showed decreased intima to media ratio with no other apparent histological differences detected at any time-point. Sex differences in lesion development further illustrate the complex nature of the *ApoE*$^{-/-}$ mouse model.

# Angiotensin levels in preterm babies

Barbara Lingwood[1], Eugenie Lumbers[2], Rebecca Dyson[3], Yvonne Eiby[1] & Ian Wright[2,3].

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**Background:** Cardiovascular compromise is common in the first 24 hours after preterm birth and is associated with increased risk of mortality and morbidity. The renin-angiotensin system plays a major role in the regulation of cardiovascular function in the adult and has been shown to be active in the fetus. Altered levels of angiotensin peptides could contribute to preterm cardiovascular compromise.

**Aims/Hypothesis:** This preliminary study aimed to determine plasma levels of angiotensin II (Ang II) and angiotensin-(1-7) (Ang-(1-7)) in the first 3 days of life in preterm infants.

**Methods:** Blood was collected from preterm infants born at 24-35 weeks gestation. Collection times included cord blood, 6h after birth, 24, 48 and 72 hours after birth. Plasma was assessed for Ang II and Ang-(1-7) levels by RIA. For analysis, babies were divided into 2 groups: very preterm – 24-28 weeks (N=45), and preterm – 29-35 weeks (N=43). Numbers were lower at individual time points as all samples were not collected in all babies.

**Results:** Ang II levels increased with post-natal age (PNA) age in both very preterm (VPT) and preterm (PT) infants. At most time points Ang II levels were higher in VPT infants than in PT infants. Ang-(1-7) also increased with PNA in VPT infants but this pattern was less evident in PT infants. There was no effect of gestational age on Ang-(1-7) levels until after 24h of age when levels were lower in the PT infants. The mean ratio of Ang-(1-7):Ang II was consistently higher in VPT infants than in PT infants in the first 48h after birth.

**Conclusions:** The vasoconstrictor action of Ang II is dependent on a functional sympathetic nervous system (SNS). In the preterm infant, where the SNS is immature, high levels of Ang II may contribute to cardiovascular compromise by acting on angiotensin type 2 receptors which are vasodilator. As Ang-(1-7) is also a vasodilator, the high Ang-(1-7):Ang II ratios will exacerbate this problem.

# NOTES

# Impact of mechanical ventilation on preterm diaphragm and protective effect of Vitamin A and Retinoic Acid

Yong Song[1], MJ Dahl[2], Kurt H. Albertine[2], J. Jane Pillow[1]

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**Background:** Ventilator-induced diaphragmatic dysfunction (VIDD) is widely described in adults, but the impact of mechanical ventilation (MV) on the preterm diaphragm is unknown. Preterm babies have inadequate body stores of vitamin A at birth resulting in increased susceptibility to diseases of the respiratory tract, severe infection, and disturbed growth. In animals, vitamin A deficiency is associated with decreased rate of protein synthesis and altered expression of contractile protein in skeletal muscle. Collectively, these findings imply that retinoid signalling may be an important regulator of muscle dysfunction of preterm development.

**Aims/Hypothesis:** We aimed to establish the impact of MV on preterm diaphragm fibre composition, protein signalling, proteolytic activity and tissue oxidation and to evaluate the effect of enteral Vitamin A and Retinoic Acid (VARA: 1:10 combination) on the development of VIDD in the mechanically ventilated preterm lamb. We hypothesized that the preterm diaphragm was susceptible to VIDD, and that VARA supplementation in ventilated preterm lambs reduced the severity of VIDD in the preterm diaphragm.

**Methods:** 131 d preterm lambs received synchronized intermittent mandatory ventilation for 3 d. Lambs were treated with daily enteral doses of 2500 IU/kg (n=6) or 5000 IU/kg (n=4) VARA during MV, or with enteral saline (n=10). Unventilated control lambs were euthanized at birth (n=7). The fetal diaphragm was collected for quantifying expression of myosin heavy chain (MHC) isoforms and atrophic genes (*MAFbx* and *MuRF1*), assessing activity of ubiquitin proteasome pathway (UPP), determining protein carbonyl content and investigating catabolic signalling (FOXO and NF-κB).

**Results:** Postnatal MV significantly decreased MHC neonatal mRNA amount, but increased MHC IIx mRNA level ($p < 0.05$). UPP activity was elevated after 3 d of MV, accompanied by increased MuRF1 expression and protein carbonyl level. VARA supplementation with 2500 IU/kg but not 5000 IU/kg decreased UPP activity and catabolic signalling (FOXO), down-regulated *MuRF1* gene expression and attenuated oxidative injury.

**Conclusions:** 3d MV likely causes preterm lamb diaphragmatic impairment, which is associated with abnormal expression of myofibrillar composition, activation of protein degradation pathway and oxidative injury. Enteral VARA is a potential therapeutic option for preterm infants with VIDD, and attenuates catabolic signaling through preventing cellular oxidative stress.

# Effects of severe intrauterine inflammation on mechanically ventilated preterm lambs

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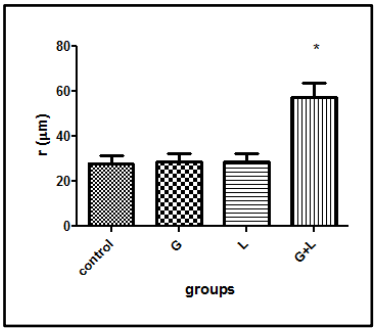
**Background:** New BPD (bronchopulmonary dysplasia), characterised by arrested alveolar development resulting in fewer and larger alveoli, is believed to be associated with intrauterine inflammation. However, the impact of intrauterine inflammation on development of new BPD has not been fully understood.

**Aims:** To determine the effects of severe intrauterine inflammation on the postnatal alveolarization in mechanically ventilated preterm lambs.

**Methods:** Time mated pregnant ewes underwent surgery at 123 DG (days of gestation, term ~147DG). Foetuses were randomly divided into 4 groups at 125 DG. One group (G group, n=4) received daily intravenous injection of G-CSF (granulocyte colony stimulating factor; 40μg/day, 105-109 DG). A second group (L group, n=4) received bolus intra-amniotic injection of LPS (lipopolysaccharide; 20mg) at 127 DG. A third group (G+L group, n=4) received both G-CSF and LPS with the same schedule as G group and L group. The control group (n=4) received sham surgery only. At 130 DG, lambs were delivered, treated with exogenous surfactant, mechanically ventilated. At 10 days after birth, lambs were euthanized and the lungs were removed, pressure fixed, and stained with Haematoxylin-Eosin, Masson-Trichrome, and Hart's elastin. Following general histological analysis, alveolar quantitative morphometry analysis was performed and secondary crest density was measured. The umbilical cords were processed for histologic analysis and scored for degree of inflammation. Data was statistically compared between 4 groups.

**Results:** All the foetuses in G+L group had necrotizing funisitis, where no foetuses had the lesion in other three groups. Surface density of alveolar walls was significantly lower, average alveolar radius was larger (figure), and numerical density of alveoli was lower in G+L group than in other groups. Secondary crest density in G+L was significantly lower than in other groups, while there was no difference in elastin content in the lung tissue between groups.




**Conclusions:** It was suggested that severe intrauterine inflammation with necrotizing funisitis induced fewer and larger alveoli in the postnatal lungs which could be caused by arrest of alveolarization.

# Partial aeration with 100% oxygen at birth increases pulmonary blood flow in aerated and non-aerated regions

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**Background:** Aeration of the lungs at birth increases pulmonary blood flow (PBF). The mechanisms behind the increase in PBF are thought to be the entry of air and removal of lung liquid, and exposure of the pulmonary microcirculation to an increase in oxygen ($O_2$) levels. Our recent studies demonstrated that aeration of the right lung resulted in increased PBF in both lungs; that is in aerated and non-aerated regions. This surprising result has led to this study investigating the relative effects of $O_2$ on the increase in PBF at birth.

**Aims/Hypothesis:** We aimed to determine how partial ventilation with 100% $O_2$ affects the PBF changes at birth using simultaneous phase contrast (PC) X-ray imaging and angiography. It was expected that 100% $O_2$ would enhance the ventilation/perfusion mismatch between aerated and non-aerated regions previously reported.

**Methods:** Newborn rabbits (n=6) were delivered near-term (~30 d GA; term ~32 d GA) and immediately PC X-ray imaged while an iodinated contrast agent was infused into the jugular vein, to visualise PBF before and then during aeration of one lung with 100% $O_2$ gas. The inspired gas was switched to air (21% $O_2$), and both lungs were then aerated. The number of visible pulmonary blood vessels, their diameter and the relative change in intensity was measured, the latter providing a relative measure of PBF.

**Results:** Aeration of one lung with 100% $O_2$ increased visible vessel number (from 29±2 to 72±4), vessel diameter (from 439±5μm to 495±20μm) and relative PBF (from 10.4±5.5% to 22.3±2.4%) in the liquid-filled and unaerated lung. There was a significant difference in vessel diameter (495±20μm vs. 619±40μm) and relative PBF (22.3±2.4% vs. 28.1±3.3%) between non-aerated and aerated lungs when only the right lung was ventilated with 100% $O_2$.

**Conclusions:** Partial lung aeration with 100% $O_2$ enhances the ventilation/perfusion mismatch between aerated and non-aerated regions in the lungs previously reported. This study enhances our understanding of the role of $O_2$ concentration in the increase in PBF at birth.

# Does an impaired antioxidant system modify the effects of neonatal hyperoxia on lung development?

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**Background:** Owing to lung immaturity, very preterm infants usually require supplemental oxygen ($O_2$), but this can alter lung development. These infants are likely to be particularly vulnerable to the damaging effects of oxidants that are produced when hyperoxic gas is inhaled as they are born with an immature antioxidant system. A major endogenous antioxidant enzyme is glutathione peroxidase 1 (*gpx1*), which is upregulated significantly in late gestation; therefore we have used *gpx1-/-* knock-out mice to model the low antioxidant status of preterm infants.

**Aims/Hypothesis:** To use a *gpx1-/-* knock-out mouse model to determine the effect of an impaired antioxidant system on lung injury induced by neonatal exposure to mild hyperoxia (40% $O_2$).

**Methods:** Both *gpx1+/+* and *gpx1-/-* neonatal mice were continuously exposed to 40% $O_2$ from birth until postnatal day 7 (P7d). Controls (CON) breathed room-air. All mice were maintained in room-air until adulthood at P56d (*gpx1+/+* CON n=16, *gpx1+/+* 40% $O_2$ n=16, *gpx1-/-* CON, n=17, *gpx1-/-* 40% $O_2$ n=16). At P56d, lung architecture was morphometrically analysed and the immune cells in the bronchoalveolar lavage fluid (BALF) were enumerated and characterised.

**Results:** At P56d the *gpx1-/-* CON group had a lower lung tissue fraction (% tissue space) than the *gpx1+/+* CON group; however no significant differences were observed in alveolar dimensions between groups. The number of immune cells in the BALF of the *gpx1+/+* 40% $O_2$ and *gpx1-/-* 40% $O_2$ groups was significantly greater than in the *gpx1+/+* CON group. Furthermore when the BALF immune cells were characterised, there was a significant increase in the proportion of lymphocytes in the *gpx1-/-* 40% $O_2$ group in comparison to the other groups.

**Conclusions:** Exposure to 40% $O_2$ led to a persistent increase in the number of immune cells in both the *gpx1+/+* and *gpx1-/-* groups. The *gpx1-/-* 40% $O_2$ group exhibited an increase in the proportion of lymphocytes in BALF, suggesting a greater vulnerability to respiratory diseases such as asthma and COPD.

# NOTES

# Macrophages prevent spontaneous preterm birth in mice

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**Background:** On-time parturition involves a coordinated progression from an anti-inflammatory to pro-inflammatory environment in gestational tissues. Macrophages are potent regulators of the maternal immune adaptations required for successful gestation and delivery.

**Aims/Hypothesis:** We hypothesized that CD11b+ monocyte/macrophages have a critical role in controlling the timing and success of birth, potentially through immune-regulatory and anti-inflammatory actions.

**Methods:** CD11b-*dtr* mice mated with wild-type FVB males were given diphtheria toxin (DT; 25 ng/g, to deplete CD11b+ cells) or PBS on gestation day (gd)16 or gd17, with or without exogenous progesterone or bone marrow-derived wild-type macrophages (n=9-12 per group). Gestation length, duration of labor, and perinatal parameters were recorded. In a second cohort, leukocytes in blood and gestational tissues were analyzed by clow cytometry 24h after DT or PBS injection, and decidual mRNA was analyzed by $RT^2$ Profiler PCR arrays.

**Results:** In CD11b-*dtr* mice given DT on gd16, CD11b+ macrophages were depleted from the myometrium, decidual tissues and placenta by 95%, 70% and 40% respectively, but were unaffected in fetal liver. Expression of several genes regulating inflammation was dysregulated in decidual tissues. CD11b+ cell depletion caused preterm delivery in CD11b-*dtr* mice, with birth 20h earlier than in control mice ($P<0.05$, Mann-Whitney U-test). The duration of active labor was increased 1.4-fold, pups weighed 15% less and their viability was poor compared with controls (26% vs 100%)(all $P<0.05$). No substantial change in the timing or characteristics of labour was seen when DT was given on gd17. Administration of exogenous macrophages, but not progesterone on gd16, alleviated prematurity and normalized labour duration in DT-treated mice, with an increased fetal survival rate of 72% (all $P<0.05$).

**Conclusions:** Acute depletion of maternal CD11b+ cells during late gestation induced preterm delivery and perinatal death, and replacement with exogenous wild-type macrophages protected mice from fetal loss. CD11b+ cells are essential for maintenance of pregnancy on gd16, but appear superfluous from gd17. These data suggest that macrophages have a physiological role in exerting anti-inflammatory effects to maintain uterine quiescence and restrain premature progression to labor. Thus, insufficient number or function of anti-inflammatory macrophages warrants investigation as a cause of preterm delivery in women.

# Expression of MicroRNAs that regulate inflammation in cord blood from preterm neonates.

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**Background:** Preterm neonates are highly susceptible to developing inflammatory morbidities which have both a short and long-term health impact. The immune basis for these outcomes remains unclear, though it is evident that preterm neonates display different responses to immune challenge clinically and *in-vitro*. MicroRNAs (miRs) are critical regulators of inflammation, however, they are not well characterised in the context of neonatal immunity. MiRs can regulate gene expression within the inflammatory Toll-like Receptor (TLR) signalling pathway through targeting the expression of various intracellular compounds and complexes. For example, miR155 is a negative regulator of the *SOCS* gene and therefore promotes an increase in cytokine production during inflammation. Alternatively, in response to inflammatory cues, *let7e* represses TLR4 expression, while miR146a and miR146b repress the TLR signalling molecule *IRAK1*; thereby, down-regulating inflammation.

**Aims/Hypothesis:** The aim of this study is to identify the differential expression of key immune miRs in cord blood of preterm and term neonates. We hypothesise that preterm miR expression will reflect a pro-inflammatory immune-regulatory bias.

**Methods:** Cord blood was collected from preterm (<37 weeks of gestational age; *n*=5) and term deliveries (*n*=7). qRT-PCR was used to characterise the expression of miRs *let7e*, miR155, miR146a and miR146b relative to RNU48 gene expression. Data was analysed with respect to gestational age.

**Results:** Preterm cord blood demonstrated decreased expression of *let7e* and increased expression of miR155 and miR146a compared to term cord blood. There was no difference in miR146b expression between the study groups.

**Conclusions:** Decreased *let7e* and greater miR155 expression suggests a decreased capacity to regulate pro-inflammatory signaling in preterm neonates. The data in this study are preliminary: further confirmatory PCR expression work of miR targets and larger sample sizes is required. However, the differences in the expression of miRs that regulate innate immune signalling between preterm and term neonates could contribute to the excess inflammatory-morbidity observed in this vulnerable population.

# Influence of human amnion epithelial cells on the fetal inflammatory response

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**Background**: Human amnion epithelial cells (hAECs) are anti-inflammatory, and can modulate the effects on lung and brain development of experimental intrauterine inflammation in preterm fetal sheep.
**Aims/Hypothesis**: We aimed to determine the effect of hAECs on the acute fetal inflammatory response to intra-amniotic injection of lipopolysaccharide (LPS).

**Methods**: Pregnant ewes underwent surgery for implantation of catheters into the amniotic cavity and a fetal jugular vein and carotid artery. After recovery (at 122 days of gestation: term is ~147 days) 2ml of saline, either with or without LPS (10 mg from *E coli*), was injected intra-amniotically; 3 ml of phosphate buffered saline, either with or without hAECs (90 million), was injected intravenously to the fetus (group sizes are in the Table). Serial blood gas and plasma samples were collected. Fixed and frozen tissue samples were collected at 48 h. Fetal lung inflammation was assessed by counting CD45+ cells using immunohistochemistry and measuring mRNA levels for pro-inflammatory cytokines interleukin (IL)-1β, IL-6 and IL-8 by qRT-PCR. The fetal systemic response was assessed by measuring hepatic mRNA levels for acute phase proteins serum amyloid A 3 (SAA3) and C-reactive protein (CRP). Data were compared by 2-way ANOVA.

**Results**: Fetal blood lactate levels at 10 h were higher in LPS than saline groups ($p<0.05$). Blood gas and metabolite levels were not different between hAECs groups.

| | Intra-amniotic Saline | | Intra-amniotic LPS | |
|---|---|---|---|---|
| | IV PBS | IV hAECs | IV PBS | IV hAECs |
| Number of subjects | 6 | 5 | 8 | 6 |
| Lung: | | | | |
| CD45+ cells/field | 1±1 | 1±1 | 27±4* | 11±1*# |
| IL-1β mRNA | 1.0±0.3 | 0.8±0.3 | 20.7±16 | 15±4 |
| IL-6 mRNA | 1.0±0.5 | 1.1±0.2 | 2.0±0.7 | 2.0±0.8 |
| IL-8 mRNA | 1.0±0.7 | 0.2±0.1 | 3.4±2.1* | 8.3±4.2* |
| Liver: | | | | |
| SAA3 mRNA | 1.0±0.8 | 0.8±0.2 | 156±51* | 98±19* |
| CRP mRNA | 1.0±0.5 | 0.8±0.1 | 1.7±0.4* | 2.2±0.4* |

*Data are mean±SEM. *p<0.05 v IA Saline. #p<0.05 v IA LPS + IV PBS.*

**Conclusions**: Intra-amniotic LPS-induced fetal lung inflammatory cell infiltration is reduced by hAECs but pulmonary and hepatic pro-inflammatory gene expression is not altered. Modulation of the fetal pulmonary developmental effects of IA LPS by hAECs does not appear to occur as a result of attenuation of the initial inflammatory response.

# NOTES

# Effect of placental restriction and late maternal methyl supplementation on immune functional outcomes in sheep

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**Background:** Fetal growth restriction, which reduces methyl donor supplies to the fetus, decreases allergy incidence later in life. Conversely, maternal dietary methyl donor supplementation in late pregnancy increases risk of allergy in offspring. We have previously reported that placental restriction (PR) impairs allergic responses in adolescent sheep (1).

**Aims/Hypothesis:** We therefore hypothesised that maternal methyl supplementation in late pregnancy would reverse effects of PR on antibody and cutaneous hypersensitivity responses to antigen sensitisation in adolescent sheep.

**Methods:** Outcomes were measured in 59 control (CON) lambs, 28 PR lambs and 25 PR lambs whose mothers were fed methyl donors (PR+METHYL; 2 g rumen-protected methionine, 300 mg folate, 1.2 g S, 0.7 mg Co/day) from d 120 of pregnancy until term delivery. We measured serum antibody (Ab) responses to immunological sensitisation with house dust mite (HDM) and ovalbumin (OVA), and subsequent wheal reactions to intradermal antigen injection.

**Results:** Birth weight was greater in CON (5.6 ± 0.2 kg) than in PR (4.6 ± 0.2 kg, P=0.002) or PR+METHYL lambs (4.2 ± 0.2 kg, P<0.001). Within multiple-birth sheep, fold-increase in OVA-specific IgE (P=0.022) was greater in PR (1.77 ± 0.16-fold increase) than in PR+METHYL (1.12 ± 0.15) sheep (P=0.013). A greater proportion of PR (78.4 ± 8.3%) than either CON (37.7 ± 8.0%, P=0.001) or PR+METHYL (33.8 ± 9.8%, P=0.001) sheep had positive (≥2-fold increase) HDM-specific IgE responses. In singletons only, PR+METHYL sheep (4.10 ± 0.54-fold increase) had greater OVA-specific $IgG_1$ responses than PR sheep (2.74 ± 0.50-fold increase, P=0.010). A greater proportion of PR+METHYL (Overall: 89.7 ± 6.3%; Singletons: 100.00 ± 0.00%) than either CON (Overall: 60.2 ± 10.8%, P=0.020; Singletons: 49.8 ± 22.8%, P=0.034) or PR (Overall: 58.3 ± 12.0%, P=0.021; Singletons: 40.6 ± 16.4%, P=0.001) sheep had positive OVA-specific $IgG_1$ responses. Fold-increases in OVA-specific IgA were greater in PR females (1.38 ± 0.10-fold increase) than in CON females (0.95 ± 0.08-fold increase, P=0.010). The proportion of skin wheal responders to OVA were lower in PR (21.8 ± 10.2%) than in CON (77.6 ± 14.8%) sheep (P=0.003).

**Conclusions:** PR increases Ab responses to sensitisation without increasing cutaneous hypersensitivity, suggesting that PR suppresses inflammatory responses to allergens downstream of an Ab response. Effects of PR on Ab responses were partially reversed by maternal methyl supplementation in late pregnancy, consistent with epigenetic mechanisms.

(1) Wooldridge AL, *et al.* (2014) *Am J Physiol: Regul Integr Comp Physiol* **5 Feb** [E-Pub]

# Characterization of $GABA_A$ receptors in the preterm brain

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**Background:** The incidence of late preterm birth is increasing in developed nations and is associated with the onset of neurodevelopmental morbidities later in life. Neurodevelopment *in utero* is heavily dependent on the fetal neurosteroid allopregnanolone, which has many functions including encouraging the fetal 'sleep' state, decreasing damaging seizures *in utero*, and promoting myelination within the brain, by acting on $GABA_A$ receptors that are located synaptically and extrasynaptically throughout the central nervous system. Whilst it is known that these receptors increase in concentration over the course of gestation, are highly sensitive to allopregnanolone, and that differing expression of these receptors has been linked with anxiety related disorders, it is unknown whether neonates born preterm have an altered expression profile of $GABA_A$ receptor subunits and whether this may be linked with the reduced myelination seen in late preterm infants.

**Aims/Hypothesis:** This project aimed to characterise the expression of key $GABA_A$ receptor subunits that control neurosteroid sensitivity in the neonatal guinea pig preterm and term brain and assess markers of stress and brain maturation.

**Methods:** Guinea pig neonates were delivered by c-section preterm (GA62) or term (GA69). Saliva was collected at 2 and 24hrs post birth. Neonatal brains (hippocampus and cerebellum) were collected 24hrs post birth. $GABA_A$ receptors were quantified by real time PCR, salivary cortisol by ELISA and myelination by immunohistochemistry (myelin basic protein).

**Results:** Cerebellar expression of the α6 and δ subunits was decreased in preterm neonates, whilst hippocampal δ subunit was increased. Preterm neonates demonstrated decreased myelination in the subcortical white matter, cerebellum and hippocampus. Cortisol concentrations 24 hours after birth were also increased in preterm neonates, however this was not the case 2 hours after birth, suggesting an inability of the preterm neonates to adapt to the *ex utero* environment.

**Conclusions:** These findings have important implications for the wellbeing of late preterm neonates as altered expression of $GABA_A$ subunits have been linked with the onset of anxiety and depressive related disorders. These observations highlight the disadvantages that preterm neonates face when born prematurely.

# What effect do antenatal steroids and magnesium sulphate have on neurotrophic factors in preterm infants?

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**Background:** Up to 50% of preterm children will experience problems with motor function, language, reading and/or speech by school age. To confer a degree of neuro-protection for preterm neonates, antenatal steroids and magnesium sulphate ($MgSO_4$) are administered to women in threatened preterm labour. Neurotrophic factors are proteins involved in neural growth, survival and differentiation, and are critically important for in-utero development of the central nervous system. The impact of antenatal steroids and $MgSO_4$ on neurotrophic concentrations remains unknown.

**Aims:** The aim was to assess levels of brain derived neurotrophic factor (BDNF), neurotrophic factor-3 (NT3) and neurotrophic factor-4 (NT4), according to gestational age, exposure to magnesium sulphate and antenatal steroid therapy.

**Methods:** Cord blood from 233 preterm infants (<37 weeks) was collected at delivery and BDNF, NT3 and NT4 levels were measured by ELISA. The impact of $MgSO_4$ was assessed only in infants born ≤30 weeks gestation (n=61).

**Results:** All neurotrophins increased across gestation. BDNF levels were higher in females than males (p=0.015) and were unaffected by antenatal steroids in both males and females. In infants ≤ 32 weeks gestation, steroid exposure did not affect NT3, but resulted in increased NT4 when birth occurred after 24 hours of steroid exposure (p=0.013). In late preterm infants (>32 weeks gestation), NT3 levels were higher in unexposed infants and those born within 24 hours of steroid exposure, compared to those born outside of 24 hours (p<0.001). NT4 levels were unaffected by steroid exposure in late preterm infants. In infants born <30 weeks gestation, an interaction effect was observed between $MgSO_4$ and steroid exposure on BDNF levels (p=0.003). BDNF levels increased within 24 hours of steroid exposure (p<0.01) and returned to baseline levels thereafter only in infants exposed to $MgSO_4$. A similar trend was observed in NT3 levels following MgSO4 exposure, while $MgSO_4$ had no effect on NT4 levels.

**Conclusions:** The transient increase in BDNF and NT3 following both $MgSO_4$ and antenatal steroid therapies, which are not observed following either therapy alone, may alter neurodevelopmental outcomes in preterm infants. The reduction in NT3 levels in later preterm infants following steroid therapy warrants further investigation.

# Magnesium for perinatal hypoxia-ischemia in term infants: A systematic review of preclinical studies

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**Background:** There is an important, unmet need to further improve the outcome of neonatal encephalopathy in term infants. It remains controversial whether $MgSO_4$ is clinically neuroprotective, and thus, it is unclear whether it would be appropriate to test $MgSO_4$ for treatment of encephalopathy in term infants. Our aim was to systematically review the preclinical evidence for neuroprotection with $MgSO_4$ before or after hypoxic-ischemic encephalopathy (HIE) in term equivalent perinatal animals.

**Methods:** Studies were characterized into those that showed improved or unaffected neural outcomes based on histological and / or behavioural assessments, and species, treatment regime (timing and dose of treatment), extent of temperature monitoring and survival time after treatment were assessed.

**Results:** We identified a total of 15 studies in near term/term animals that assessed histological and / or behavioural outcomes after treatment with $MgSO_4$ for HIE. Most of the studies (12/15) were performed in neonatal rats (P7-21), with 2 studies in near term sheep and 1 in term piglets. 7/15 (47%) studies reported improved outcomes; all but one reported histological outcome only. One study reported improved outcomes when $MgSO_4$ was combined with oxygen derived free radical scavengers; the effects of $MgSO_4$ alone were not assessed. The dose of $MgSO_4$ varied widely between studies (range: 72-500 mg/kg). All gave treatment systemically, in either single or multiple doses. 4/7 studies showing neuroprotection did not monitor temperature, 2/7 monitored ambient temperature and 1/7 intermittently monitored core temperature. In studies showing no effect of $MgSO_4$, 2/7 did not monitor temperature, 3/7 monitored ambient temperature and 2/7 studies, both in large animals, controlled core temperature throughout the experiment. No rodent studies directly measured brain or body temperature. 14/15 studies examined short to medium survival times (<1-13 days). One study examined outcomes after 35 days, this was the only study to report improved sensorimotor function.

**Conclusions:** Over half of available studies found no improvement with $MgSO_4$ in models of HIE, including most of the large animal studies. The lack of adequate control of brain and body temperature measurements in studies suggesting protection, limited long term neuropathological and behavioral assessments and lack of effect in large animal 'translational models' of perinatal HIE indicate that $MgSO_4$ cannot be recommended at present for clinical trial for HIE in term infants.

# Neuroprotective effects of hAECs following neonatal hyperoxia

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**Background:** Exposure to high concentrations of oxygen (hyperoxia) during the neonatal period is associated with increased morbidity and mortality. Consequences of hyperoxia in the neonate include bronchopulmonary dysplasia and brain injury. Human amnion epithelial cells (hAECs) possess stem cell-like properties, are immunomodulatory and have been shown to decrease hyperoxia-induced lung damage.

**Aims/Hypothesis:** This study aimed to assess the efficacy of hAECs in reducing brain injury in a neonatal mouse model of hyperoxia. We hypothesized that hAECs would reduce injury via immunomodulation.

**Methods:** Newborn mice were separated into two cohorts, normoxia (control; 21% $O_2$) and hyperoxia (85% $O_2$). These conditions were maintained from postnatal day (PND) 1 to 14. Mie received either $1.5x10^6$ hAECs intraperitoneally (IP) on PND 5, 6 and 7 (for a total of $4.5x10^6$) or 50µl of saline IP. Brains were collected for histopathology, protein and RNA analysis.

**Results:** Hyperoxia significantly increased cell death within the cortex compared to normoxic controls, as measured by the number of caspase-3 positive cells. This effect was significantly ameliorated by administration of hAECs. Hyperoxia caused a significant increase in the number of inflammatory cells (Iba-1 immunoreactivity) within the cortex; and a significant increase in the inflammatory cytokines IL-17, TNF-$\alpha$, IFN and IL-6 within the whole brain homogenate, which were all decreased following hAEC treatment. Analysis of the white matter revealed a decrease in myelin density in hyperoxic mice within the periventricular and subcortical white matter and this was ameliorated in the mice that received hAECs.

**Conclusion:** Hyperoxia significantly increased cell death, inflammation and white matter injury, this effects were ameliorated by the administration of hAECs. This study demonstrates that hAECs may have a role in preventing hyperoxic brain injury during the neonatal period.

# Localisation of human amnion epithelial cells to the cerebral white matter is not required for neuroprotection

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**Background:** Resuscitation of preterm infants in the delivery room can inadvertently cause lung injury and resultant cerebral white matter (WM) damage, due to altered cerebral haemodynamics and translocation of inflammatory mediators. Human amnion epithelial cells are anti-inflammatory, protect against lung injury, and can reduce fetal brain inflammation associated with experimental chorioamnionitis.

**Aims/Hypothesis:** We hypothesized that hAECs would mitigate cerebral inflammation and injury caused by neonatal resuscitation/ventilation of preterm lambs.

**Methods:** Two groups of lambs (0.85 gestation) were used: (i) Ventilated lambs (Vent; n=8) were injuriously ventilated (target tidal volume 15 mL/kg, no positive end-expiratory pressure) for 15 min and subsequently ventilated for 105 min; (ii) hAEC lambs (Vent+hAEC; n=7) were similarly ventilated but received intravenous and intratracheal administration of $9x10^7$ CFSE-labelled hAECs (total $18x10^7$) soon after birth. Brains were collected for assessment of inflammation and blood brain barrier integrity using qRT-PCR (interleukin (IL)-1β, IL-6 and IL-8, and tight junction protein, occludin) and immunohistochemistry (anti-Iba-1 and anti-sheep serum antibodies). CFSE-labelled cells were quantified in cerebral WM within the frontal and parietal lobes. Student's t-test was used to compare groups.

**Results:** In the subcortical WM, IL-6 mRNA levels and microglial density were lower in Vent+hAEC lambs compared to Vent lambs ($p=0.03$ and $p=0.01$, respectively). IL-1β, IL-6 and IL-8 mRNA levels in the periventricular WM were higher in Vent+hAEC lambs than Vent lambs ($p<0.05$). Occludin mRNA levels in the periventricular WM tended higher in Vent+hAEC lambs compared to Vent ($p=0.06$). Vascular leakage was lower in Vent+hAEC lambs than Vent lambs ($p=0.046$). In 3 of 7 Vent+hAEC animals, hAECs were visualised in the cerebral WM. The average number of cells found in both lobes combined was 7 ± 4 hAECs/total periventricular WM area and 11 ± 4 hAECs/total subcortical WM area. No relationship was found between the presence of hAECs and other variables.

**Conclusions:** Administration of hAECs soon after birth appears to modulate cerebral inflammation induced by injurious resuscitation/ventilation in preterm lambs. Human AECs can gain access to the brain in preterm lambs with resuscitation/ventilation-induced lung and brain injury.

# NOTES

# Epigenetic basis of lung and brain damage in chronically ventilated preterm lambs

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**Background:** Respiratory failure and mechanical ventilation (RFMV) predisposes preterm infants to neonatal chronic lung disease. Though MV is life-saving, its side effects, such as neurodevelopmental impairment, often are life-long. The mechanisms by which impairment occurs are unknown and therapies are unavailable. We recently showed that MV of preterm lambs is associated with shifts in apoptosis and proliferation in the lung and brain, as well as genome-wide histone hypoacetylation, in both lung and brain. These changes are also associated with altered levels of insulin-like growth factor-1 (IGF-1) in both organs. IGF-1 may be relevant because regulation of expression of this morphogen is epigenetic.

**Objective:** Test the role of epigenetics by blocking histone deacetylation in preterm lambs with RFMV.

**Design/Methods:** Preterm lambs, treated with antenatal steroids and postnatal surfactant, were managed by (1) MV, (2) MV+valproic acid (VPA; non-specific histone deacetylase inhibitor, HDACi), (3) MV+trichostatin A (TSA; specific HDACi), or (4) high-frequency nasal ventilation (HFNV; positive gold-standard for alveolar formation) (n=4). Treatment was daily (im) for 3d. Histone modifications were measured by immunoblot.

**Results:** MV for 3d is associated with alveolar simplification, decreased apoptosis of mesenchymal cells, increased proliferation of mesenchymal cells, and higher levels of IGF-1 in the lung. In the brain of the same preterm lambs, diffuse brain damage is evident as a slightly more apoptosis of neurons and glia, and lower levels of IGF-1. At 3d of MV plus daily VPA or TSA, genome-wide acetylation of histone3/lysine14 (H3K14ac) and H3K18ac was significantly greater in the lung (~30%; $p<0.05$) and brain (white and gray matter; ~25%; $p<0.05$) compared to MV. HDACi treatment improved alveolarization, reduced IGF-1 levels, and improved respiratory gas exchange ($p<0.05$) compared to MV alone. Lung hyperacetylation, and structural and functional parameters were similar between the MV+HDACi-treated and nasal HFV groups. Initial data suggest that HDACi also led to less apoptosis and more proliferation, and more IGF-1 level, in the brain.

**Conclusions:** Blocking histone deacetylation, using HDACi, during MV preserves genome-wide hyperacetylation of histones in the lung and brain. We propose that epigenetics is a common mechanism that links evolving lung and brain injury in preterm neonates that have RF that requires MV. We speculate that clinical approaches that preserve histone hyperacetylation may reduce the incidence and/or severity of lung and brain injury, and perhaps improve long-term outcomes. (HL110002, HL062875, HL07744)

# Adaptation of the cardiac renin-angiotensin system (RAS) to extrauterine life in term and preterm piglets

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**Background:** The transition from birth to extrauterine life requires the heart to change from a circuit where the ventricles are working in parallel and the right ventricle is dominant, to one working in series in which the left ventricle becomes the powerhouse. The term heart generally transitions effectively but the preterm heart has greater difficulty. There is evidence that the RAS is involved in the left ventricular hypertrophy that is part of the transition process.

**Aims:** To determine if there are changes in cardiac RAS expression between birth and 6h of age in term piglets, and to investigate if similar changes occur in preterm piglets.

**Methods:** Piglets were delivered by caesarean section at term (113/115 days) and prematurely (97days). An additional group of premature piglets were exposed to a clinically relevant dose of maternal glucocorticoids. Left and right ventricular tissue was collected at birth (0h) or after 6h of extrauterine life. Q-PCR was used to determine the relative gene expression of components of the cardiac RAS including *AGT, ATP6AP2 {(Pro) renin receptor}, ACE1, ACE2, AGTR1, AGTR2 and Mas R.*

**Results:** At birth in the left ventricle *AGT* expression was lower in preterm piglets than term and was higher in glucocorticoid treated preterm piglets than untreated preterms. In all groups expression at 6h was higher than at birth. Expression of the *(Pro) renin receptor* was also upregulated at 6h compared to birth across all groups. Expression of other genes will be discussed.

**Conclusions:** These results demonstrate that preterm hearts at birth have lower expression of a number of genes thought to be involved in cardiac development. Glucocortiocoid exposure increases the expression of these genes at the time of birth. This may contribute to the beneficial effects of GC treatment on cardiovascular function. Upregulation of these genes at 6h suggests a significant role in cardiovascular transition.

# Premature birth and metabolic outcomes in sheep

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**Background:** Preterm birth affects 8.3% of Australian babies, with 80% (>20,000 pa) born moderate-late preterm at 32-36 weeks completed gestation[1], and with adverse consequences for health into adulthood. This includes an increased risk of developing diabetes in those born preterm, including after moderate-late preterm birth[2-4]. The underlying mechanisms for effects of preterm birth on diabetes and insulin action are poorly understood, with evidence for insulin resistance mostly in childhood[5], and effects on insulin secretion not characterised.

**Hypothesis:** Induced moderate-late preterm birth in sheep will impair glucose homeostasis in postnatal life.

**Methods:** Ewes were induced to deliver moderately preterm at 133 days gestation (term ~147 days) by maternal administration of epostane, and with maternal administration of antenatal betamethasone on days 131 and 132 of gestation. Glucose tolerance and insulin secretion were assessed by intravenous glucose tolerance test (0.25 g glucose.kg$^{-1}$,IVGTT), in progeny delivered preterm or at term, in a pilot study at ~ 6 weeks of age (Study 1, n= 2 preterm, 9 term-delivered) and in a larger cohort at ~14 months of age (Study 2, n=16 preterm, 17 term-delivered).

**Results:** In the pilot study of lambs at 6 weeks of age, fasting glucose before IVGTT was higher in preterm than term-born lambs (P=0.049), with a similar trend for glucose concentrations throughout the entire IVGTT (P=0.053), and glucose tolerance did not differ between groups. In Study 2, preterm birth also did not alter glucose tolerance during IVGTT, but altered fasting glucose in a sex-specific manner (gestational age*sex interaction P=0.004). Preterm birth increased fasting glucose in young adult females (Term: 3.88 ± 0.12 mM; Preterm: 4.31 ± 0.10 mM; P=0.020), but not in males (Term: 4.08 ± 0.08 mM; Preterm: 3.89 ± 0.09 mM; P=0.130).

**Conclusions:** Delivery at moderate-late preterm gestational age appears to induce mild deficits in glucose handling in growing lambs, with stronger evidence for this in young adult females. This has not yet progressed to frank glucose intolerance in healthy young adults. We are now investigating insulin secretion and sensitivity.

1. Li Z et al. Australia's mothers and babies 2011. Canberra: AIHW National Perinatal Epidemiology and Statistics Unit, 2013; 2. Lawlor D et al. *Diabetologia* 49: 2614-7, 2006; 3. Kaijser M et al. *Diabetes* 58: 523-6, 2009; 4. Crump C et al. *Diabetes Care* 34: 1109-13, 2011; 5. Tinnion R et al. *Arch Dis Child*, 2013.

# The role of fibroblast growth factors in inflammation-induced fetal lung maturation

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**Background:** Exposure to inflammation during gestation increases the risk of preterm birth. Paradoxically, inflammation during gestation reduces the risk of respiratory distress syndrome in newborns, due to increased pulmonary surfactant production. We have evidence to suggest that inflammation-induced increases in surfactant protein production in pulmonary epithelial cells is mediated by a soluble factor secreted by pulmonary fibroblasts (Dolan *et al*., PSANZ 2014).

**Aims/Hypothesis:** We hypothesised that known mesenchymal-epithelial signalling factors, such as fibroblast growth factor (FGF)-7, FGF-10 and hepatocyte growth factor (HGF), mediate the inflammation-induced increase in surfactant production. Our aim was to measure FGF-7, FGF-10 and HGF mRNA levels in mouse lung fibroblasts exposed to lipopolysaccharide (LPS).

**Methods:** Primary fetal mouse lung fibroblasts (E18.5; n=1) and a neonatal mouse pulmonary fibroblast cell line (Mlg; n=2) were cultured in the presence of LPS (from *E. coli* 055:B5; 1μg/ml) or saline. Twenty-four hours after LPS exposure, cells were collected and RNA was extracted. Expression of FGF-7, FGF-10 and HGF was assessed using qPCR.

**Results:** Expression of FGF-7, FGF-10 and HGF were >20-fold higher in LPS-exposed primary fetal lung fibroblasts compared to saline controls. Expression of FGF-7 was 2.5-fold higher in LPS-exposed Mlg cells, compared to control, but there was no difference in FGF-10 or HGF mRNA levels.

**Conclusions:** Preliminary results suggest FGF-7 as a candidate soluble factor that may signal preterm lung epithelial cells to increase surfactant expression in response to pulmonary inflammation. Studies are underway to further investigate the role of FGF-7 in mediating the inflammation-induced increase in surfactant production by the preterm lungs.

# NOTES

# Regulation of renin angiotensin system (RAS) pathways in the human decidua

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**Background:** Pregnancy outcome is influenced, in part, by the sex of the fetus. The maternal decidua has its own renin angiotensin system (RAS), which is thought to regulate placental development and uteroplacental blood flow. We have previously demonstrated that decidual renin mRNA (*REN*) abundance is greater in women carrying a female compared to a male fetus, and that this sex difference is maintained for up to 48 h *ex vivo*.

**Aims/Hypothesis:** In this study, we explored whether the sex of the fetus also influences the regulation of decidual RAS expression by cyclic AMP (cAMP), a known stimulator of renal renin.

**Methods:** Decidual explants were either treated with vehicle or 300 mM cAMP and were cultured for 24 and 48 hours; gene expression of all RAS components and prorenin, angiotensin II (Ang II) and angiotensin 1-7 (Ang 1-7) protein was measured using RT-qPCR, ELISA and radioimmunoassay, respectively.

**Results:** cAMP had no affect on *REN* expression in decidual explants, since *REN* abundance was still greater in decidual explants from women carrying a female compared to a male fetus ($P<0.01$). Interestingly, cAMP decreased prorenin levels in the supernatant if the fetus was female ($P<0.01$), *i.e.,* prorenin levels were no longer sexually dimorphic.

cAMP treatment altered fetal sex specific differences in other RAS genes; it abolished sex differences in angiotensinogen (*AGT*), prorenin receptor (*ATP6AP2*) and Mas receptor (*MAS1*) genes and reversed the pattern of angiotensin converting enzyme 2 (*ACE2*) and Ang II type 1 receptor (*AGTR1*) expression.

The presence of male cells was confirmed by measuring expression of the *SRY* gene, as a means to detect fetal cells in the maternal decidua.

**Conclusion:** The present study demonstrates that the decidual RAS response to cAMP is influenced by fetal sex. This may have significant implications for our understanding of the sex specific differences in pregnancy outcome.

# Vitamin D and IGF gene expression in first trimester and term placentae

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**Background:** Vitamin D deficiency is prevalent, affecting 30-60% of Australians, and is associated with pregnancy complications, such as preeclampsia and gestational diabetes. These pregnancy complications are characterised by impaired placental development in early gestation. Placental development is mediated by the insulin-like growth factor (IGF) family which is also involved in the vitamin D pathway.

**Aims/Hypothesis:** This project aims to determine the differential expression of vitamin D and IGF genes in the human placenta across gestation. We hypothesise that expression vitamin D and IGF pathway genes is different between early and late first trimester as well as at term.

**Methods:** Placentae were collected from uncomplicated term pregnancies and from first trimester terminations. First trimester placentas were divided into those collected before and after 9 weeks gestation. Real time qPCR was used to quantify expression of vitamin D receptor (*VDR*), vitamin D hydroxylase (*CYP2R1*), *IGF1*, *IGF2*, *IGF1R* and *IGF2R*.

**Results:** Placental expression of *VDR* and *CYP2R1* were 10-fold and 2-fold higher, respectively, at term compared to first trimester ($P<0.001$). *IGF2* was highest in first trimester, with early and late first trimester having 3-fold ($P=0.004$) and 1.8-fold ($P=0.038$) higher expression, respectively, compared to term. *IGF1R* expression was 1.6-fold higher in first trimester than at term ($P<0.001$). This suggests that *IGF2* and *IGF1R* are more abundant in first trimester, as the IGFs regulate early placental development; whereas at term *VDR* and *CYP2R1* appear to be more highly required in later gestation, with *CYP2R1* increasing local placental vitamin D ($25(OH)D_3$) production. Expression of *CYP2R1* also increased 1.8-fold across first trimester ($P=0.020$). There was also a strong correlation between *VDR* and *IGF2* in both first trimester ($R=0.665$, $P=0.007$) and at term ($R=0.764$, $P=0.001$). *VDR* expression was also positively correlated with *IGF2R* in both first trimester ($R=0.857$, $P<0.001$) and at term ($R=0.603$, $P=0.013$) and *IGF1* in first trimester only ($P=0.035$).

**Conclusions:** Placental expression of both vitamin D related and IGF genes are altered across gestation, with correlations between *VDR* and *IGF*s evident. The altered expression of *CYP2R1* during early gestation may be due to an increased need for vitamin D as the placenta changes from a hypoxic to a normoxic environment. Further research will investigate the role of vitamin D on placental function during pregnancy and whether IGF and vitamin D pathways interact.

# Placental GR isoform expression varies in relation to gestational age at delivery in a sex specific manner

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**Background:** During human pregnancy, conditions may arise that promote excess fetal exposure to maternal glucocorticoids, which in turn can significantly impact the lifelong health of the offspring. We have previously identified sex-specific differences in the fetal response to cortisol. Specifically, male fetuses appear to induce a state of glucocorticoid resistance in an environment of excess glucocorticoids while females remain sensitive to changes in glucocorticoid concentration. Our recent studies suggest that this differential response to cortisol is driven by differences in glucocorticoid receptor (GR) protein function. We have discovered there are actually 12 different GR isoforms in the human term placenta, 5 in the placental vascular endothelium and 4 in cord blood immune cells that could regulate the level of cellular sensitivity to glucocorticoids.

**Aims/Hypothesis:** Our hypothesis is that sex-specific differences in cortisol sensitivity in fetal-placental tissues are controlled by the interaction of functional GRα A with other GR isoforms. The cell-specific differences in GR isoform expression control differences in glucocorticoid-regulated gene expression and subsequently, affect growth in utero and early childhood development. In this current study we have examined whether there are differences in GR isoform expression in relation to fetal sex and gestational age at delivery.

**Methods:** Human placentae were collected within 45 mins of delivery from term and preterm pregnancies. Preterm was classified as less than 37 completed weeks of gestation. Placental tissue was snap frozen, protein extracted from cytoplasmic and nuclear fractions and Western blot performed using a total GR antibody that detects all GR isoforms.

**Results:** Preterm placentae (24-36 weeks gestation, n=21) had 12 isoforms of the GR similar to term placentae (n=50). However, the concentrations of each isoform varied in relation to gestational age and sex. Female preterm placentae had significantly greater concentrations of GRα A, GRα C, GR P, GR A, GRα D1 and D3 expression relative to female term placentae. In preterm male placentae, there were greater concentrations of GRα C, GR A, GRα D1, D2 and D3 relative to male term placentae. When examining the entire population, birthweight was positively correlated with the expression of GRβ, GR P and GRα D2 in males and GR P and GRα D2 in females.

**Conclusions:** The data indicates there are significant alterations in GR isoform expression as gestation progresses. Differences in sensitivity to cortisol between preterm males and females may be conferred by sex-specific expression of several GR isoforms including GRα A, GRβ, GR A and GRα D 1-3. The complex interaction between these different GR isoforms and betamethasone exposure and the impact on glucocorticoid regulated pathways are the focus of our current research.

# Can neonatal exendin-4 prevent diabetes after IUGR?

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**Background:** Intrauterine growth restriction (IUGR) in humans impairs adult insulin sensitivity and secretion, and hence increases the risk of diabetes [1, 2]. Similarly, IUGR due to placental restriction (PR) in sheep and rats impairs postnatal insulin action, by reducing β-cell function and insulin sensitivity [3-5]. A potential intervention is neonatal exendin-4 (EX-4) treatment, which prevents diabetes in the PR rat [6] and increases insulin secretion with reduced food intake and insulin sensitivity during treatment in twin IUGR lambs [7]. However, whether insulin action in adulthood is improved by neonatal EX-4 after IUGR in sheep remains unknown.

**Hypothesis:** Neonatal EX-4 treatment of the PR sheep normalises insulin secretion, insulin sensitivity, and glucose tolerance in adulthood.

**Methods:** Singleton progeny of control ewes (CON; n=6F, 4M), PR ewes (PR; n=13F, 7M), and PR progeny that were treated with EX-4 (PR+EX-4; 1 nmol/kg s.c., daily from d1 to d16 of age; n=11F, 7M) were weighed at birth and throughout life. Glucose tolerance and insulin secretion were assessed as young adults (~339d) by intravenous glucose tolerance test (0.25 g glucose.kg$^{-1}$, IVGTT), and insulin sensitivity by hyperinsulinaemic euglycaemic clamp (120 min; 2 mU insulin kg$^{-1}$min$^{-1}$).

**Results:** Fasting glucose tended to be higher in PR+EX-4 than CON (P=0.054; CON: 3.50±0.17 mM, PR: 3.61±0.12 mM, PR+EX-4: 3.92±0.13 mM). Glucose tolerance (area under the glucose curve, $AUC_{glucose}$; CON: 247±29 mM.min, PR: 326±21 mM.min, PR+EX-4: 289±22 mM.min) was higher in PR than CON (p=0.035), and not different in PR+EX-4 compared to CON or PR (each p>0.2). Despite this, $AUC_{insulin}$ (p=0.914) and insulin secretion ($AUC_{insulin}$:$AUC_{glucose}$, p=0.601) did not differ between groups. Insulin secretion was higher in males than females (p=0.016). Insulin sensitivity was similar between groups (p=0.8) and higher in females than males (p=0.041).

**Conclusions**: Glucose tolerance was impaired in young adult PR sheep, compared to controls. Neonatal exendin-4 treatment appears to partially normalise glucose homeostasis.

1. Jaquet D et al (2000) J Clin Endocrinol Metab 85: 1401-6.
2. Newsome CA et al (2003) Diabet Med 20: 339-48.
3. Gatford KL et al (2008) Endocrinology 149: 5118-27.
4. De Blasio MJ et al (2012) Endocrinology 153: 2142-51.
5. Simmons RA et al (2001) Diabetes 50: 2279-86.
6. Stoffers DA et al (2003) Diabetes 52: 734-40.
7. Gatford, KL et al (2013) PLoS One 8: e56553.

# NOTES

# Does IUGR have an effect on nephrogenesis in the developing human kidney?

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**Background:** Over the past decade it has become well recognised that IUGR is a contributing factor to a reduced nephron endowment at birth and it is also linked to subsequent renal dysfunction. The majority of these studies have been conducted in animal models. In this study we further characterise the effects of IUGR on nephrogenesis in the developing human kidney.

**Aims/Hypothesis:** It was hypothesised that nephrogenesis is compromised in the developing IUGR fetal kidney. The specific aims were to examine the effect of IUGR in the developing human kidney on the timing of nephrogenesis, width of the nephrogenic zone and on the number of glomerular generations formed within the cortex.

**Methods:** Kidneys were collected at autopsy (following written consent from the parents) from fetuses ranging in age from 20 weeks to 40 weeks. Portions of the kidneys were embedded in paraffin, sectioned at 5 µm, and stained with haematoxylin and eosin. The number of glomerular generations formed within the kidneys was assessed using a medullary ray glomerular counting method and where nephrogenesis was ongoing, the width of the nephrogenic zone was measured using image analysis.

**Results:** This study is still ongoing. To date, the preliminary findings have shown, as expected, that the nephrogenic zone width decreases in both IUGR and non-IUGR kidneys with increasing gestational age. The timing of the cessation of nephro-genesis was variable in IUGR and non-IUGR kidneys; for example, nephrogenesis was observed to be complete as early as 32 weeks gestation in some kidneys and was still ongoing at 37 weeks gestation in other kidneys. The findings to date, suggest that nephrogenesis is delayed in the IUGR kidneys with a reduced number of glomerular generations formed within the cortex compared to age-matched non-IUGR infants. However, the timing of nephrogenesis appeared to be extended in the IUGR kidneys with nephrogenic zone width greater than age-matched non-IUGR control kidneys at the later time points in gestation.

**Conclusions:** The preliminary findings in autopsied human kidneys demonstrate that IUGR impacts on the number of glomerular generations formed within the kidney and on kidney maturation.

# Preliminary observations on the effect of maternal health on fetal body and kidney growth in Indigenous women

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**Background:** Small babies have an increased risk of chronic disease in adult life. Animal studies have shown that maternal renal dysfunction affects fetal renal development. Renal disease is ten times more common in Indigenous people and Indigenous women are more likely to have small babies.

**Aims:** To find associations between markers of maternal renal, inflammatory, cardiovascular and metabolic health and fetal body weights and kidney volumes in a pregnant Indigenous population as part of the Gomeroi Gaaynggal NHMRC funded

**Methods:** 127 pregnant Indigenous women were studied. Fetal weights and combined kidney volumes were determined by ultrasound; biochemical variables were measured by New England Pathology and grouped into renal (6), inflammatory (6), cardiovascular (6) and metabolic (3) factors. Regression equations were used. Gestational age (days) was included in each equation as it is the major factor affecting fetal weight and kidney volume. No variable was considered to have an effect if it did not contribute significantly to the regression equation.

**Results:** Women were 25.8 ± 0.5 years of age; 6 women had low GFRs, 12 had microalbuminuria (albumin:creatinine > 3.4 mg/mmol); 19 had proteinuria (urinary protein:creatinine > 30 mg/mmol) and 76 had high CRP levels (> 3.0 mg/L).
*Renal health:* Only maternal GFR influenced fetal weight (partial correlation = 0.644, P = 0.005); kidney volume was negatively influenced by maternal urinary Na/K (partial correlation = -0.28, P = 0.048).
*Inflammation:* No inflammatory marker influenced fetal weight but maternal IgA levels did affect combined kidney weight (partial correlation = 0.62, P = 0.016)
*Cardiovascular:* There were no effects on fetal weight or kidney volume.
*Metabolic:* Maternal plasma cholesterol was associated with fetal weight (partial correlation = 0.289, P = 0.02).

**Conclusions:** Maternal Cystatin C levels (a measure of GFR) and plasma cholesterol was positively associated with fetal weight but not fetal kidney volume, which was negatively influenced by urinary Na/K. Both maternal plasma cholesterol and urinary Na/K ratios are likely to depend on maternal diet. IgA nephropathy is a well-known cause of chronic renal disease but why maternal IgA levels influence fetal kidney growth cannot be explained.

# Permanent nephron loss following neonatal acute kidney injury in male offspring, does this lead to chronic kidney disease?

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**Background:** Acute kidney injury (AKI) in the neonatal period has an association with increased risk of chronic kidney disease (CKD) and related pathologies later in life. A key contributor to neonatal AKI is a birth complicated by asphyxia. Using an established model of birth asphyxia in the spiny mouse we identified significant disruption to the architecture of the kidney, 24 hours after insult. In addition, we found that supplementing the maternal diet with creatine could prevent kidney injury[1].

**Aims/Hypothesis: 1.** To determine whether disruption to kidney architecture persists into childhood, as a permanent loss of nephrons, thereby increasing the risk of CKD**. 2.** Whether the apparent protective capacity of creatine is long lasting.

**Methods:** Pregnant spiny mice were maintained on normal chow or chow supplemented with 5% w/w creatine from mid-gestation. At term, pups were delivered by caesarean section or subjected to intrauterine asphyxia. Post mortems were conducted at 33 days postnatal age (P33), and kidneys collected for stereological analysis of nephron endowment, or at 85 days of age, before which assessment of urinary osmolality and electrolyte levels (P48 & P72), and conscious glomerular filtration rate (GFR; P85) were undertaken.

**Results:** At P33 glomerular number was reduced by ~20% in birth asphyxia males (P=0.03), but not females. Nephron endowment of male offspring of creatine-supplemented dams remained at control levels. Whilst results are preliminary, GFR measured in conscious spiny mice appears unaffected in male offspring (control 261.4 ± 12.4 µl/min (n=5); birth asphyxia 262.4 ± 45.33 (n=3) at P85. Analysis of urinary osmolality and electrolyte balance are still underway.

**Conclusions:** Structural disturbances observed at 24 hours after birth asphyxia persist as a reduction in nephron number in male offspring. This nephron deficit does not appear to affect basal renal function. Whether, this reduction in nephron endowment, when coupled with adverse environmental factors such as obesity, leads to an increased risk of developing CKD in this model remains to be determined. Maternal creatine supplementation prevents birth asphyxia induced renal injuries and may prove useful in reducing the incidence of neonatal AKI.

[1]Ellery et al. Pediatric Research. 2012;73:201

# NOTES

# NOTES

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