

# Fetal and Neonatal Physiological Society

## 36th Annual Meeting

September 27-30, 2009  
UCLA Conference Center  
Lake Arrowhead, California, USA



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# Minutes of the FNPS Annual General Meeting

Maastricht, The Netherlands, Wednesday 25 June 2008

1. Meeting minutes. The minutes of the Sendai meeting held in Japan in 2007 were accepted with no amendments.
2. Changes to the Board and words of thanks:
  - (i) Professor Jan Nijhuis assumed the Chair of the FNPS Board Members. He gave thanks on behalf of the Society to Dr Laura Bennet for her tireless work serving as the Society's previous Chair.
  - (ii) Dr Dino Giussani assumed the position of Scribe for the Society. Professor Jan Nijhuis expressed thanks to Professor Bill Parer for his efficient work serving as the previous Scribe.
  - (iii) Professor Gerry Visser and Professor Richard Harding stepped down as FNPS Board Members. They were replaced by Dr Lucy Green and Professor Luc Zimmermann. Professor Jan Nijhuis expressed thanks to Professors Visser and Harding for their tenure on the Board.
  - (iv) Professor Jan Nijhuis also thanked the rest of the organizing and scientific committees of the Maastricht meeting for making it such a successful event.
3. FNPS Archives. It was suggested that Liggins' notes should be kept at The Liggins Institute. The possibility of housing a complete set of FNPS abstract books at the University of Cambridge was discussed. Dr Giussani was to explore this possibility and report back to the Board Members.

4. Future meetings:

- (i) 2009. Lake Arrowhead, USA, 27-30 September;
- (ii) 2010. Possibility of joint meeting held in UK with The DOHaD Society, The Physiological Society and The Blair Bell Research Society.
- (iii) 2011. Australia/New Zealand

5. Student Prizes:

Best oral presentations

- (i) A. Baburamani. Abstract 106. Monash University, Australia.
- (ii) P. Pathipati. Abstract 804. University of Melbourne, Australia.

Best poster presentations

- (iii) H. Torrance. Abstract P205. UMCU, Utrecht, The Netherlands.
- (iv) H. Heineman. Abstract P220. Maastricht University, The Netherlands.

Respectfully submitted,

A handwritten signature in cursive script, appearing to read 'Dino A. Giussani', with a long horizontal flourish extending to the right.

Dino A. Giussani, PhD

*FNPS Scribe*

# Meeting at a Glance

## September 27 (Sunday)

4:00-6:30 PM: Check-in time.

5:00-6:30 PM: Welcome reception

6:30-8:00 PM: Dinner

8:00-9:30 PM: Dawes Lecture, to be delivered by Dr. Lawrence Longo

## September 28 (Monday)

8:00-10:00 AM: Oral Session 1 (Hypoxia 1)

10:30 AM-noon: Oral Session 2 (Hypoxia 2)

Noon-1:30 PM: Lunch

1:30-3:00 PM: Oral Session 3 (Clinical/Translational 1)

3:00-3:30 PM: Break

3:30-5:00 PM: Oral Session 4 (Pulmonary and Vascular Biology)

6:30-8:00 PM: Dinner

## September 29 (Tuesday)

8:00-10:00 AM: Oral Session 5 (Perinatal Programming)

10:30 AM-noon: Oral Session 6 (Neurobiology and Endocrinology)

Noon-1:30 PM: Lunch

1:30-5:00 PM: **Sporting Event!**

6:30-8:00 PM: Dinner

8:00-10:00 PM: Poster Discussion

## September 30 (Wednesday)

8:00-10:00 AM: Oral Session 7 (Clinical/Translational 2)

10:30 AM-noon: Oral Session 8 (Endocrinology)



36<sup>th</sup> Annual Meeting of the Fetal and Neonatal Physiological Society

# Geoffrey Dawes Lecture

“Geoffrey S. Dawes and the Rise  
of Fetal and Neonatal Physiology”

Lawrence Longo, M.D.  
Director, Center for Perinatal Biology  
Distinguished Professor Basic Sciences and  
Gynecology and Obstetrics  
Loma Linda University  
School of Medicine

**SESSION I: (Hypoxia 1)**  
**Chair: Charles Ducsay**

Monday September 28<sup>th</sup>

- 8:15am – 8:30a.m      Abstract 1:  
Can Insulin Like Growth Factor-1 Improve White Matter Protection with Delayed Cerebral Hypothermia?  
S George, J Davidson, L Bennet, J Bouwmans J, TR Gunn TR, AJ Gunn
- 8:30am – 8:45am      Abstract 2:  
Maternal Creatine Supplementation During Pregnancy Protects the Newborn Spiny Mouse Brain from Intrapartum Hypoxia  
David W Walker, Zoe Ireland, Margie Castillo-Melendez, Hayley Dickinson & Rod Snow
- 8:45am – 9:00am      Abstract 3:  
Blood Nitrite and Iron-Nitrosyl Hemoglobin Concentrations in Response to Acute Hypoxia in the Fetal Sheep.  
Arlin B. Blood, Hobe Schroeder, Gordon G. Power, Lawrence D. Longo.
- 9:00am – 9:15am      Abstract 4:  
Electrocortical (ECOG) Activity in the Ovine Fetus with Placental Embolization and Chronic Hypoxemia  
Ashley E. Keen, Martin G. Frasch, Melissa A. Sheehan, Robert Gagnon and Bryan S. Richardson
- 9:15am - 9:30am      Abstract 5:  
Maternal Melatonin Administration Provides Neuroprotection in Late-Gestation Fetal Sheep in Response to Umbilical Cord Occlusion  
Yawno T, Castillo-Melendez M, Jenkin G, Wallace EM, Walker DW, Miller SL
- 9:30am – 9:45am      Abstract 6:  
Maternal Melatonin Protects Against the Developmental Programming of Cardiovascular Disease in Hypoxic Pregnancy  
J.A. Hansell, E.J. Camm, H.G. Richter, E.A. Herrera, C.E. Blanco, E. Villamor & D.A. Giussani
- 9:45am – 10:00am      Abstract 7:  
Blockade of SOC And ROC Channels Attenuates the Hypoxia-Induced Pulmonary Hypertension *In Vivo* And Small Pulmonary Arteries Contractile Status *Ex Vivo*.  
D Parrau, G Ebensperger, C Ulloa, F Moraga, R Riquelme, M Díaz, C Fierro, P Silva, E Herrera, R Rojas, AJ Llanos, and VR Reyes

**CAN INSULIN LIKE GROWTH FACTOR-1 IMPROVE WHITE MATTER PROTECTION WITH DELAYED CEREBRAL HYPOTHERMIA?**

George S, Davidson J, Bennet L, Bouwmans J, Gunn TR, Gunn AJ.

Department of Physiology, The University of Auckland, New Zealand.

**Introduction:** Cooling the brain after perinatal hypoxia-ischemia can significantly reduce injury and improve long-term neurological outcome. However, both clinically and experimentally, protection is only partial. One likely reason is the progressive reduction in effectiveness of cooling with greater delay after injury. Potentially combining hypothermia with another agent could further improve outcome of delayed treatment. We and others have previously shown that insulin-like growth factor-1 (IGF-1) can reduce neuronal loss and promote oligodendrocyte survival and myelination after ischemia; in particular IGF-1 promotes proliferation of oligodendrocytes and other glia, and thus might counterbalance the glial suppression seen with hypothermia.

**Material and Methods:** Unanesthetized near-term fetal sheep *in utero* were subjected to 30 minutes of cerebral ischemia. Fetuses were then randomized to receive either cooling from 5.5 to 72 hours (n = 12) or an infusion of 3 µg of IGF-1 intracerebroventricularly from 4.5 to 5.5h plus cooling from 5.5 to 72h (n=8), or sham cooling plus sham infusion (n=12), or sham ischemia (n=5). Cooling was induced by circulating cold water through a coil around the fetal head. The water temperature was titrated to reduce fetal extradural temperature from 39.1 +/- 0.1 °C to between 30 and 33 °C, while maintaining esophageal temperature >37°C. Fetuses were killed after 5 days for histological assessment. Data are mean±SD.

**Results:** 30 min of cerebral ischemia was associated with severe white matter damage in a watershed pattern, with loss of CNPase positive intragyral oligodendrocytes compared with sham controls (380±138 vs 1180± 152 cells/field, p<0.001). Delayed hypothermia alone reduced this loss (847±297 cells/field, p<0.01 vs ischemia alone). There was no significant difference between hypothermia alone and hypothermia plus IGF-1 infusion (1015±211 cells/field, NS). Ischemia was associated with marked activated caspase-3 expression in white matter (216±41 vs 19±18 cells/field, p<0.001). Delayed hypothermia was associated with a reduction in activated caspase 3 positive cells (116±81 cells/field, p<0.05), with no significant difference between hypothermia alone and hypothermia plus IGF-1 infusion (91±27 cells/field, NS).

**Conclusions:** Partial white matter protection with delayed, moderate cerebral hypothermia is associated with reduced caspase-3 activation after 5 days recovery. There was no apparent further improvement from combination therapy with delayed IGF-1 infusion, potentially suggesting that hypothermia and IGF-1 are at least in part protecting oligodendroglia through overlapping anti-apoptotic mechanisms.

## Maternal creatine supplementation during pregnancy protects the newborn spiny mouse brain from intrapartum hypoxia

<sup>1</sup>David W Walker, <sup>1</sup>Zoe Ireland, <sup>1</sup>Margie Castillo-Melendez, <sup>1</sup>Hayley Dickinson & <sup>2</sup>Rod Snow

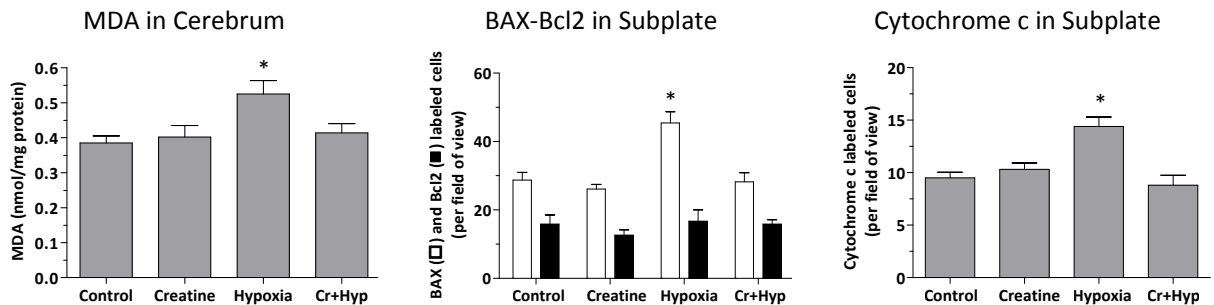
<sup>1</sup>Fetal & Neonatal Research Group, Department of Physiology, Monash University, Clayton, Victoria; <sup>2</sup>School of Exercise & Nutrition Sciences, Deakin University, Burwood, Victoria, AUSTRALIA

[david.walker@med.monash.edu.au](mailto:david.walker@med.monash.edu.au)

**Introduction:** The creatine-phosphocreatine shuttle is essential for the maintenance of cellular ATP, particularly under hypoxic conditions when respiration may become anaerobic. Creatine synthesis by the fetus appears to be attained late in gestation, and until then the fetus appears to rely on creatine from the maternal pool (Ireland et al., 2009). Using a model of intrapartum hypoxia in the precocial spiny mouse, we have previously shown that a maternal diet supplemented with 5% creatine monohydrate from mid-gestation improves survival and postnatal growth in offspring (Ireland et al, 2008). The present study assessed the potential for maternal creatine supplementation to protect the fetal brain from the effects of intrapartum hypoxia.

**Methods:** Birth hypoxia was induced in fetal spiny mice at 38 days gestation (term is 39 days) by isolating the pregnant uterus in a warm saline bath for 7-8 mins. The pups were then resuscitated and cross-fostered to a nursing dam.

**Results:** At 24 h after birth-hypoxia, the brains of surviving offspring showed significant increases in lipid peroxidation as measured by the amount of malondialdehyde (MDA, nmol/mg protein). There were also significant increases in the number of cells expressing the pro-apoptotic protein BAX and cytoplasmic cytochrome c in the cortical subplate, thalamus and piriform cortex. When pregnant dams were fed the creatine supplemented diet, the increase in malondialdehyde, BAX and cytoplasmic cytochrome c was almost completely prevented so that they were not different from caesarean-delivered neonates (see figure).



**Discussion:** This study provides evidence that the neuroprotective capacity of creatine in the hypoxic perinatal brain involves abrogation of lipid peroxidation and apoptosis, and the maintenance of mitochondrial function. Further investigation into the long-term development and behavioural outcomes of such neonates is warranted.

### References:

Ireland Z et al (2009) Developmental changes in the expression of creatine synthesizing enzymes and creatine transporter in a precocial rodent. *BMC Developmental Biology* (in press)

Ireland Z et al (2008) Maternal creatine: does it reach the fetus and improve survival after an acute hypoxic episode in the spiny mouse (*Acomys cahirinus*)? *Am J Obstet Gynecol.* 198:431.e1-431.e6.

## BLOOD NITRITE AND IRON-NITROSYL HEMOGLOBIN CONCENTRATIONS IN RESPONSE TO ACUTE HYPOXIA IN THE FETAL SHEEP.

Arlin B. Blood<sup>1,2</sup>, Hobe Schroeder<sup>2</sup>, Gordon G Power<sup>2</sup>, Lawrence D Longo<sup>2</sup>.

<sup>1</sup>Department of Pediatrics, Division of Neonatology, and <sup>2</sup>Center for Perinatal Biology, Loma Linda University School of Medicine, Loma Linda, CA.

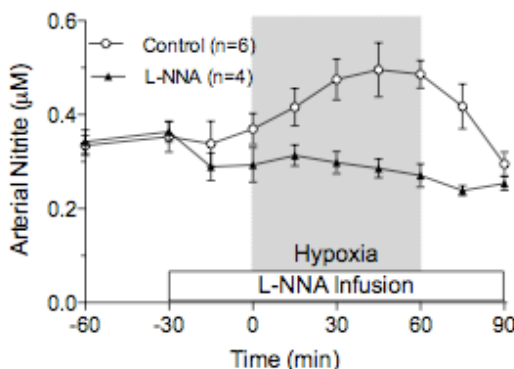
**Background:** Recent evidence indicates that nitrite ( $\text{NO}_2^-$ ) in blood, once considered a biologically inert byproduct of nitric oxide (NO) metabolism, can be reduced back to NO by reaction with deoxyhemoglobin. This reaction may result in the local production of NO in circulating blood and in hypoxic tissues, serving to increase  $\text{O}_2$  delivery by vasodilation. Recently, we have reported that, *in vitro*, fetal deoxyhemoglobin produces NO from nitrite at a rate twice that of the adult<sup>1</sup>. Little is known, however, about the effect of hypoxia on circulating nitrite concentrations in the fetus, or the effect of nitrite metabolism on vascular tone in the fetus during hypoxic stress. The objective of this work was to measure the effect of acute hypoxia on blood nitrite concentrations in the chronically instrumented fetal sheep. We further investigated the influence of nitric oxide synthase (NOS) activity on blood nitrite concentrations by repeating the experiments following NOS blockade.

**Material/Methods:** We sampled blood from the brachial artery of chronically instrumented near-term fetal lambs while the ewe was subjected to a 60 min hypoxic insult (fetal arterial  $\text{PO}_2$  10-12 Torr) without (n=6) or with (n=4) blockade of endogenous NO production by L-NNA (45  $\text{mg} \oplus \text{kg}^{-1}$  i.v. bolus, followed by 25  $\text{mg} \oplus \text{kg}^{-1} \oplus \text{hr}^{-1}$  infusion). Fetal mean arterial blood pressure and heart rate were recorded continuously. Blood samples were collected at predetermined timepoints during all periods, and nitrite concentrations were measured via triiodide chemiluminescence.

**Results:** Fetal whole blood nitrite concentrations increased from a baseline of  $340 \pm 40 \text{ nM}$  (mean  $\pm$  SEM) to a peak of  $490 \pm 80 \text{ nM}$  after 40 min of acute hypoxic insult ( $p < 0.01$ ), and correlated inversely with arterial  $\text{PO}_2$  ( $r^2 = 0.64$ ). No significant increase in nitrite concentration occurred during hypoxia during L-NNA infusion, with whole blood nitrite concentrations decreasing from L-NNA infusion decreased whole blood nitrite concentrations from  $360 \pm 20 \text{ nM}$  to  $290 \pm 30 \text{ nM}$  ( $p < 0.05$ ) before hypoxia. There was no significant change in nitrite concentrations during hypoxia while L-NNA was infused.

**Conclusions:** Acute hypoxia results in increased whole blood nitrite concentrations. L-NNA infusion blocks this increase, suggesting the nitrite is a byproduct of NOS activity. The extent to which circulating nitrite is also a source of NO in the fetus remains to be determined.

**References:** 1. Blood AB, *et al.* Am J Physiol Heart Circ Physiol. 2009 Feb;296:H237-46.



## ELECTROCORTICAL (ECOG) ACTIVITY IN THE OVINE FETUS WITH PLACENTAL EMBOLIZATION AND CHRONIC HYPOXEMIA

Ashley E. Keen<sup>1</sup>, Martin G. Frasch<sup>1</sup>, Melissa A. Sheehan<sup>1</sup>, Robert Gagnon<sup>1</sup> and Bryan S. Richardson<sup>1</sup>.

<sup>1</sup>Ob/Gyn & Phys/Pharm, University of Western Ontario, London, Ontario, Canada.

**Objective:** Placental insufficiency leading to chronic fetal hypoxemia and growth restriction may result in aberrant brain development with and/or due to altered behavioural state activity. We therefore hypothesized that placental embolizations with chronic hypoxemia in the ovine fetus will result in altered ECoG state activity as a reflection of and/or contributing to brain dysfunction.

**Methods:** Fetal sheep were chronically instrumented with arterial catheters and ECoG electrodes and then either embolized (EMB group, n=5) daily between 117-134 days of gestation (dGA, term 145) to maintain an arterial oxygen saturation (O<sub>2</sub> Sat) of 20-40%, or only given saline solution (Control group, n=5). ECoG activity was analyzed at 128-129 dGA and related to corresponding O<sub>2</sub> Sat and to brain/body weight ratios as a measure of growth restriction at animal sacrifice on dGA 131-134. ECoG amplitude and spectral edge frequency criteria were used to determine the incidence of low-voltage/high frequency (LV/HF), high-voltage/low frequency (HV/LF) and indeterminate voltage/frequency (IV/F) state activity, along with duration of state transition times.

**Results:** Mean O<sub>2</sub> Sat measured 46 ±1% (SEM) for the control group and 29 ±5% for the EMB group and for individual animals showed an inverse relationship to the brain/body weight ratio at animal sacrifice,  $r = -0.69$  ( $p < 0.05$ ). While the incidence of LV/HF and HV/LF did not differ, with controls at 54 ±3% and 29 ±2% and EMBs at 39 ±6% and 29 ±5%, respectively, the time spent in IV/F was increased in the EMB group at 28 ±6% vs 11 ±2% ( $p < 0.05$ ). Further, with decreasing O<sub>2</sub> Sat the incidence of IV/F increased,  $r = -0.81$  ( $p < 0.05$ ) and LV/HF decreased,  $r = 0.64$  ( $p < 0.05$ ). The state transition times from HV/LF to LV/HF were significantly shorter than that from LV/HF to HV/LF for the control group at 72 ±6 vs 100 ±7 s, respectively ( $p < 0.05$ ), but this was not seen for the EMB group at 81 ±8 vs 89 ±14 s, respectively (NS), where transition times were much more variable.

**Conclusions:** Placental embolizations with chronic hypoxemia in the ovine fetus results in an increase in indeterminate ECoG activity and altered state transition times indicating a degree of brain dysfunction. This may in turn have implications for growth processes given the likely importance of behavioural state activity in support of the brain's early development.

## MATERNAL MELATONIN ADMINISTRATION PROVIDES NEUROPROTECTION IN LATE-GESTATION FETAL SHEEP IN RESPONSE TO UMBILICAL CORD OCCLUSION

Yawno T<sup>1,2</sup>, Castillo-Melendez M<sup>2</sup>, Jenkin G<sup>3</sup>, Wallace EM<sup>1</sup>, Walker DW<sup>2</sup>, Miller SL<sup>1,2</sup>  
Departments of Obstetrics and Gynaecology<sup>1</sup>, Physiology<sup>2</sup> and Monash Immunology and Stem Cell Laboratories<sup>3</sup>, Monash University, Victoria, 3800, Australia

**Introduction:** Intermittent asphyxial and hypoxic episodes may occur *in utero* and, if the period of asphyxia is prolonged or severe, can result in serious neonatal morbidity including seizure disorders and cerebral palsy. We have previously shown that asphyxia induced by umbilical cord occlusion (UCO) in the late gestation ovine fetus leads to cell death, predominantly in the cortex, hippocampus, thalamus and cerebellum (1), and leads to oxidative stress and the generation of reactive oxygen species in these brain areas (2). We have investigated whether the pineal hormone melatonin, a direct and indirect antioxidant which can be safely administered to the mother, reduces the damage to the fetal brain which occurs in response to asphyxia.

**Methods:** Pregnant ewes underwent surgery at ~125 days gestational age (GA) for insertion of fetal and maternal catheters, and for placement of an inflatable occluder around the umbilical cord. In 10 fetuses (at ~130 days GA) asphyxia was induced by UCO for 10 min, with the ewes receiving either intravenous melatonin (1 mg bolus and 1 mg/h for 2 h; n = 5) or vehicle (1% ethanol in saline; n = 5), commencing 1 h before occlusion. The results were compared to those in vehicle infused ewes with fetal sham cord occlusion (n = 5). Brains were obtained at 48 h after UCO or sham occlusion to determine cell morphology in the subventricular region of the cerebrum, hippocampus and the cerebellum; regions that have been shown to be susceptible to increased brain injury following 10 min of complete UCO in fetal sheep.

**Results:** Hypoxemia, acidemia, hypertension and bradycardia produced by cord occlusion were similar in the melatonin and vehicle treated groups. Cord occlusion resulted in reduced expression of myelinated axons, visualized using CNPase antibody, in the subventricular white matter and subcallosal bundle. Melatonin infusion abolished this decrease. UCO caused activation of glial fibrillary acidic protein (GFAP), an astrocyte marker, in the granular layer and white matter tract of the cerebellum. Astrocytes appeared activated with multiple thickened processes; however, following melatonin infusion, astrocytes appeared to be in a resting state with thin/less processes. Lectin staining also showed that UCO produced an increase in inflammatory cells, with infiltration of macrophages from the vasculature to the purkinje cell layer and the granular layer in the cerebellum. No such inflammatory response was observed when melatonin was infused.

**Summary:** Melatonin treatment results in a decreased inflammatory response, and protection of oligodendrocytes thus preventing demyelination in brain areas that are vulnerable to hypoxic-ischemic brain damage. Melatonin may be a safe and effective treatment for use in pregnancies where there is an increased risk of fetal or intrapartum asphyxia and subsequent brain damage.

### References:

- 1) Castillo-Melendez et al 2004 *Pediatr Res* 55:864-871
- 2) Miller et al 2005 *Dev Neurosci* 27: 200-210

## MATERNAL MELATONIN PROTECTS AGAINST THE DEVELOPMENTAL PROGRAMMING OF CARDIOVASCULAR DISEASE IN HYPOXIC PREGNANCY

J.A. Hansell<sup>1</sup>, E.J. Camm<sup>1</sup>, H.G. Richter<sup>1</sup>, E.A. Herrera<sup>1</sup>, C.E. Blanco<sup>2</sup>, E. Villamor<sup>2</sup> & D.A. Giussani<sup>1</sup>

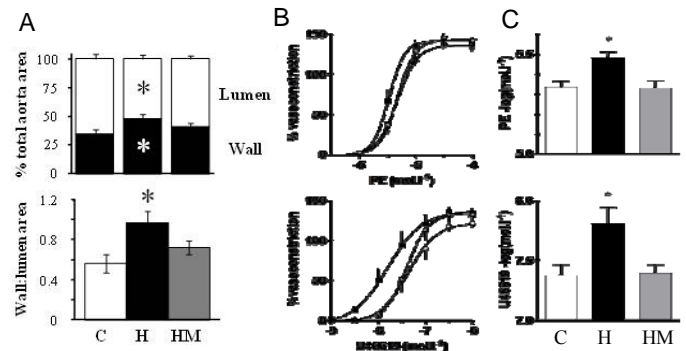
<sup>1</sup>*Department of Physiology Development & Neuroscience, University of Cambridge, United Kingdom*

<sup>2</sup>*Department of Paediatrics, Maastricht University, The Netherlands*

**Objective:** We put forward the hypothesis that oxidative stress in the fetal cardiovascular system underlies the molecular basis via which prenatal hypoxia contributes to the developmental programming of cardiovascular disease. Using an integrative approach at the isolated organ, cellular and molecular levels, we investigated the longitudinal effects on the cardiovascular system of the offspring at the end of gestation and adulthood of maternal treatment with melatonin during hypoxic pregnancy in the rat. Melatonin is widely recognised as a potent antioxidant and one of the few hormones that crosses the placenta unaltered.

**Methods:** On pregnancy day 15, 84 Wistar rats (n=14 per group) were divided into control (21% O<sub>2</sub>), hypoxic (10% O<sub>2</sub>) or undernourished (35% reduction in food intake) pregnancy, with and without maternal melatonin (5µg.ml<sup>-1</sup> drinking water). Undernourished groups served as pair-fed controls for the hypoxia-induced reductions in maternal food intake. On day 20, half of the dams were anaesthetised, the pups and placentae dissected, weighed and fixed or frozen. The remaining dams were allowed to deliver. At birth, litters were culled to 8 (4 males, 4 females) and mother and pups maintained in normoxia. Following weaning, offspring were maintained until adulthood. At 4 months, following euthanasia, organs were fixed or frozen. Fixed tissue was used for histology. Pro- and anti-oxidant protein expression was determined by Western blot. Mesenteric vessels from adults were isolated for wire myography. To control of sex and within litter variation, 1-2 male offspring from any litter for any variable measured were taken.

**Results:** Relative to controls, the reduction in birth weight (H: -9±1%, U: -11±1%, mean±S.E.M, P<0.05) and catch up growth to four months (fractional growth: H: +8±2%, U: +11±2%, P<0.05) were similar in offspring from hypoxic and undernourished pregnancy. Maternal melatonin significantly increased the placental expression of catalase and MnSOD and improved birth weight in undernourished (UM: -4±1%, P<0.05) but not hypoxic (HM: -9±1%) pregnancy. Hypoxic but not undernourished pregnancy resulted in thickening of the fetal aortic wall (Fig. 1A) with no morphological alterations to the fetal heart. In marked contrast, by adulthood, offspring of hypoxic but not undernourished pregnancy showed dilated cardiomyopathy with increased mesenteric vasoconstrictor reactivity to PE and U46619 (Fig.1B&C), but no changes in aortic structure. Maternal melatonin relieved all of the adverse cardiovascular consequences in fetal and adult offspring of hypoxic pregnancy.



**Figure 1.** Mean ± SEM for: (A) areas of the fetal aorta either as a % of the total vessel area or the wall: lumen area ratio; (B) the concentration response curves; and (C) the sensitivity (PD<sub>2</sub>) in response to PE or U46619 in mesenteric arteries from 4-month-old adults. (C, control or circles; H, hypoxic or squares; HM, hypoxia+melatonin or triangles; n=7 per group). \*P<0.05 vs C ANOVA + Tukey test.

**Conclusions:** Developmental hypoxia has differential effects on the cardiovascular phenotype of fetal and adult offspring, but maternal treatment with melatonin protects against both. The data support that oxidative stress in the fetal cardiovascular system underlies the molecular basis via which prenatal hypoxia contributes to the developmental programming of cardiovascular disease.

*Supported by The British Heart Foundation, BBSRC, The Royal Society.*

**BLOCKADE OF SOC AND ROC CHANNELS ATTENUATES THE HYPOXIA – INDUCED PULMONARY HYPERTENSION *IN VIVO* AND SMALL PULMONARY ARTERIES CONTRACTILE STATUS *EX VIVO*.**

<sup>a</sup>Parrau D, <sup>a</sup>Ebensperger G, <sup>a</sup>Ulloa C, <sup>f</sup>Moraga F, <sup>c</sup>Riquelme R, <sup>b</sup>Díaz M, <sup>a</sup>Fierro C, <sup>g</sup>Silva P, <sup>a,d,h</sup>Herrera E, <sup>a</sup>Rojas R, <sup>a,d,e</sup>Llanos AJ, <sup>a</sup>Reyes VR.

<sup>a</sup>Laboratorios FFDD y Bioquímica y Biología Molecular de la Hipoxia, <sup>b</sup>Escuela de Obstetricia, Facultades de Medicina y <sup>c</sup>Ciencias Químicas-Farmacéuticas, <sup>d</sup>INCAS, Universidad de Chile; <sup>e</sup>Universidad de Tarapacá, <sup>f</sup>Universidad Católica del Norte, Chile; <sup>g</sup>Universidad Cayetano Heredia, Perú; <sup>h</sup>Department of Physiology, Development & Neuroscience, University of Cambridge, UK.

**Introduction.** Pulmonary circulation responds to hypoxia with vasoconstriction and remodeling with the result of pulmonary hypertension. Calcium entry into the pulmonary vascular myocyte is essential for both vasoconstriction and vascular remodeling. Studies in cultured adult vascular smooth muscle cells from rodents and humans, suggest that store operated channels (SOC) and receptor operated channels (ROC) (1), two functional calcium channels, could contribute to these processes. Nevertheless there are no studies about their role in the regulation of the pulmonary circulation in newborn animals. We examined the role of these channels in high altitude newborn lambs both *in vivo* and *ex vivo*. **Material and methods.** Ten newborn lambs of 11 – 14 days old, gestated and born at Putre, at 3600 m, were used in this study. In six animals, we placed Swan-Ganz and polyvinyl catheters into the pulmonary artery, aorta and inferior cava vein respectively. All experiments were based on a 3-h protocol divided into 1-h of basal, 1-h of hypoxemia, and 1-h of recovery, in the presence of the SOC channel inhibitor, 2-aminoethoxydiphenylborinate (2-APB, 8 mg.kg<sup>-1</sup> i.v.) or its vehicle (DMSO: saline 1:10). During the study, we measured pulmonary artery and systemic pressure (PAP, SAP), heart rate (HR), cardiac output (CO) and blood gases. Four non-instrumented animals were studied *ex vivo*. Small pulmonary arteries were isolated for wire myography studies and we performed concentration response curves of 2-APB or the ROC channel inhibitor SKF-96365. **Statistical analysis.** two ways ANOVA & a Neumann – Keuls test; p<0.05.

The Faculty of Medicine Ethics Committee of the University of Chile approved all experimental procedures. **Results.** The infusion of 2-APB reduced PAP and pulmonary vascular resistance in basal conditions and during the superimposed episode of hypoxia, without modifying HR, CO, SAP or systemic vascular resistance. In contrast, the neonates infused with the vehicle had the further increase in PAP with the superimposed hypoxia, as described before in these lambs (2). In isolated pulmonary arteries precontracted with KCl or ET-1, incubation with 2-APB elicited a relaxation of 15% and 40% respectively, whilst SKF-96365 at the highest concentration assayed (100 µM) reached a 100 % relaxation in both KCl and ET-1 precontracted vessels. **Conclusion.** ROC and SOC channels are important in the control of the pulmonary artery pressure and the hypoxic pulmonary vasoconstriction in the high altitude newborn lambs. Supported by FONDECYT 1080663-Chile.

References:

- 1.- Abramowitz J & Birnbaumer L. FASEB. J. 23: 297 – 328. 2009
- 2.- Herrera EA et al. Am. J. Physiol. 292:R2234-R2240.2007.

**SESSION II: (Hypoxia 2)**  
**Chair: Anibal Llanos**

Monday September 28<sup>th</sup>

- 10:30am – 10:45am      Abstract 8:  
Differential Role of A1-Adrenergic Receptor Subtypes in Developing Cerebral Arteries  
Ravi Goyal, Ashwani Mittal, Nina Chu, and Lawrence D Longo
- 10:45am – 11:00am      Abstract 9:  
The Long Term Functional Outcome of Birth Asphyxia in Spiny Mice  
Udani Ratnayake, Lisa Hutton and David Walker
- 11:00am - 11:15am      Abstract 10:  
Vitamin C Prevents Baroreflex and Vasoconstrictor Dysfunction in the Adult Rat Induced by Chronic Fetal Hypoxia  
Andrew D Kane, Emilio A Herrera, Emily J Camm, and Dino A Giussani
- 11:15am – 11:30am      Abstract 11:  
Maternal Vitamin C Administration Improves Cognitive Function in Adulthood following Prenatal Hypoxia  
EJ Camm and DA Giussani
- 11:30am – 11:45am      Abstract 12:  
A Fetal Brain Inflammatory Response to Repetitive Umbilical Cord Occlusions (UCO) with Worsening Acidosis in the Ovine Fetus near Term  
Andrew Prout, Martin Frasch, Rob Hammond, Michael Ross, and Bryan S.Richardson
- 11:45am – 12:00pm      Abstract 13:  
Effect of Acute Hypoxia on the Motor Activity of the 10-and 14- Day Chick Embryo.  
M V Nechaeva., and IG Vladimirova.

**DIFFERENTIAL ROLE OF  $\alpha_1$ -ADRENERGIC RECEPTOR SUBTYPES IN DEVELOPING CEREBRAL ARTERIES**

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**Introduction:** The relative abundance of  $\alpha_1$ -adrenergic receptors in the cerebrovasculature suggests that vascular tone, and thus cerebral blood flow, is regulated in part by this rich adrenergic innervation.  $\alpha_1$ -adrenergic receptors are of three subtypes,  $\alpha_{1A}$ -,  $\alpha_{1B}$ -, and  $\alpha_{1D}$ -, which differ in molecular structure and tissue distribution. The subtypes also differ in their biologic function, although this area is poorly understood. Moreover, the extent to which these isoforms are differentially expressed and regulated in fetal and adult cerebral vasculature is not known. Thus, we tested the hypothesis that the  $\alpha_1$  adrenergic receptor subtypes ( $\alpha_{1A}$ -,  $\alpha_{1B}$ -,  $\alpha_{1D}$ -) function differently in cerebral arteries of the fetus, as compared to the adult. We quantified the expression levels of the several  $\alpha_1$ -receptor subtypes and their role in cerebral arterial contractility. **Methods.** In middle cerebral arteries from fetal and adult sheep, we measured the contractile responses and  $[Ca^{2+}]_i$  to the  $\alpha_1$ -AR agonist phenylephrine (PHE,  $10^{-5}$  M), in presence and absence of specific subtype blockers. **Results.** In fetal cerebral arteries, expression levels of each  $\alpha_1$ -AR subtype was considerably less than that of adult. In fetus, each subtype specific blocker, 5-MU ( $\alpha_{1A}$ ), CEC ( $\alpha_{1B}$ ) and BMY-7378 ( $\alpha_{1D}$ ) inhibited to a degree greater degree PHE-induced tension and  $[Ca^{2+}]_i$ , compared to adult. Although in arteries of both age groups, 5-MU and CEC reduced Ins(1,4,5) $P_3$  responses to PHE ~35%, BMY-7378 showed no significant decrease in Ins(1,4,5) $P_3$  responses. Also in fetal arteries, the PHE-induced increase in activated ERKs was blocked by CEC and BMY-7378, but not by 5-MU or WB-4101. **Conclusions.** In both fetus and adult,  $\alpha_{1A}$ - and  $\alpha_{1B}$ -AR appear to mediate  $\alpha_1$ -adrenergic-Ins(1,4,5) $P_3$  responses. In the fetus in contrast,  $\alpha_{1D}$ -AR appear to activate the MAPK/ERK1/2 cascade by non-Ins(1,4,5) $P_3$ -dependent mechanisms. In the immature organism, relative deficiency of specific  $\alpha_1$ -AR subtypes, and their differing signaling pathways, may be critical factors associated with dysregulation of cerebral blood flow.

**THE LONG TERM FUNCTIONAL OUTCOME OF BIRTH ASPHYXIA IN SPINY MICE****Udani Ratnayake**, Lisa Hutton and David Walker

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**Background:** Perinatal asphyxia plays a role in the aetiology of cerebral palsy and other disorders which present as cognitive and behavioural deficits in children. Birth asphyxia can result in long-term neurological alterations in various brain regions, of varying degree, depending on the severity and duration of the insult. The aim of this study was to determine whether a model of birth asphyxia in a small, precocial rodent – the Spiny Mouse (*Acomys cahirinus*) - results in deficits comparable to those seen in humans up to the pubertal stage of development.

**Methods:** Birth asphyxia or C-section delivery (control group) was performed in late gestation spiny mice at 37 days gestation (term is 39-40 days). Asphyxia was induced by placing the uterus containing the fetuses in a 37°C saline bath for 7.5 minutes from the time of maternal death. After a recovery period of 1 h pups were cross-fostered to surrogate nursing dams. Behavioural testing (open field, Rotarod, Novel Object Recognition Test [NORT]) was done from 1-30 days of age, when the pups were killed humanely and the brains retrieved for structural analysis.

**Results:** Open field testing revealed no difference in spontaneous locomotor activity between C-section delivered and asphyxiated pups at any age. However, on postnatal day 4 asphyxiated pups travelled a greater proportion of the total distance in the central zone of the field, and showed a reduction in the number of bouts of grooming. Asphyxiated pups also showed a shorter latency to fall from the rotarod, decreased improvement on the rotarod task after a delay between trials, and a decrease in the discrimination index as assessed by NORT, compared to C-section delivered pups. Histology and immunohistochemical assays identified irregular nuclear fragmentation and/or condensed chromatin particularly in cortical grey matter, and decrease of oligodendrocyte number and dis-organisation of myelinated processes in the corpus callosum and external capsule.

**Discussion:** Near-term birth asphyxia in the precocial spiny mouse resulted in persistent deficits in motor coordination, memory and learning and higher order functions, although gross motor abnormalities were not apparent. The model also produced persistent neurological alterations in the cortical grey matter, although further investigation needs to be conducted for other brain regions. This model of birth asphyxia thus produces persisting functional deficits that are comparable with the subtle deficits, such as behavioural difficulties and underachievement at school that can be seen in children who experienced perinatal asphyxia.

## VITAMIN C PREVENTS BAROREFLEX AND VASOCONSTRICTOR DYSFUNCTION IN THE ADULT RAT INDUCED BY CHRONIC FETAL HYPOXIA

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Whilst it is accepted that chronic intrauterine hypoxia can predispose to cardiovascular disease in later life, the mechanisms are not well understood.<sup>1</sup> The programming effects of developmental hypoxia may be due to the excessive generation of reactive oxygen species (ROS) in the fetal circulation. Therefore, we investigated the consequences of chronic fetal hypoxia on the ability of the adult offspring to regulate blood pressure in the rat. Further, to test the hypothesis that oxidative stress is the mechanism underlying the programming effects of developmental hypoxia, we determined whether treatment with the antioxidant vitamin C could reverse the hypoxia-induced effects.

**Methods:** Thirty female Wistar rats were randomly subjected to either normoxia (21% O<sub>2</sub>, N, n=8), normoxia + vitamin C (N+C, n=6), hypoxia (14% O<sub>2</sub>, H, n=9) or hypoxia + vitamin C (H+C, n=7) from day 6 to day 20 of pregnancy. Vitamin C was administered daily in the maternal drinking water (5 mg ml<sup>-1</sup>). Maternal weight, food and water intake were recorded daily. On day 22 the dams delivered spontaneously and the litters were reduced to 8 pups. At 4 months, one male from each litter was subjected to assessment of the cardiovascular system *in vivo*. Under halothane anaesthesia, the left femoral artery and vein were catheterised. Halothane was withdrawn and anaesthesia maintained on intravenous urethane. Following stabilisation individual baroreflex responses were generated by increasing bolus doses of phenylephrine, 0.5-80 µg i.a., and of sodium nitroprusside, 1-80 µg i.a. Responses were fitted to sigmoidal curves:  $HR = HR_{min} + (HR_{min} + HR_{max}) / (1 + 10^{((Mid-point-MAP) * Gain coefficient)})$ . Baroreflex gain was calculated as  $Gain = ((HR_{min} + HR_{max}) * Gain coefficient) / 4$ .<sup>2</sup>

**Results:** Maternal food and water intake and maternal weight gain during pregnancy were similar between groups. Chronic hypoxia did not alter baroreflex set-point (Figure 1A). However, vitamin C reduced baroreflex set-point in both normoxic and hypoxic pregnancy (Fig 1A, p<0.05). Hypoxia significantly increased baroreflex gain, which was reversed by treatment with vitamin C (Fig 1B, p<0.05). In addition, hypoxia attenuated the pressor response to increasing bolus doses of phenylephrine (Fig 1C, p<0.05). Similarly, this was reversed with vitamin C.

**Conclusion:** Chronic fetal hypoxia altered the arterial baroreflex and pressor responses to phenylephrine in adult offspring. Vitamin C prevented these effects supporting the hypothesis that ROS and oxidant tone during gestation may programme the regulation of blood pressure in adult life.

*Supported by the BBSRC, BHF, The Royal Society and the Frank Edward Elmore Trust*

1. Williams, S. J., Campbell, M. E., McMillen, I. C. & Davidge, S. T. Differential effects of maternal hypoxia or nutrient restriction on carotid and femoral vascular function in neonatal rats. *Am J Physiol Regul Integr Comp Physiol* **288**, R360-367 (2005).
2. McDowall, L. M. & Dampney, R. A. L. Calculation of threshold and saturation points of sigmoidal baroreflex function curves. *Am J Physiol Heart Circ Physiol* **291**, H2003-2007 (2006).

## MATERNAL VITAMIN C ADMINISTRATION IMPROVES COGNITIVE FUNCTION IN ADULTHOOD FOLLOWING PRENATAL HYPOXIA

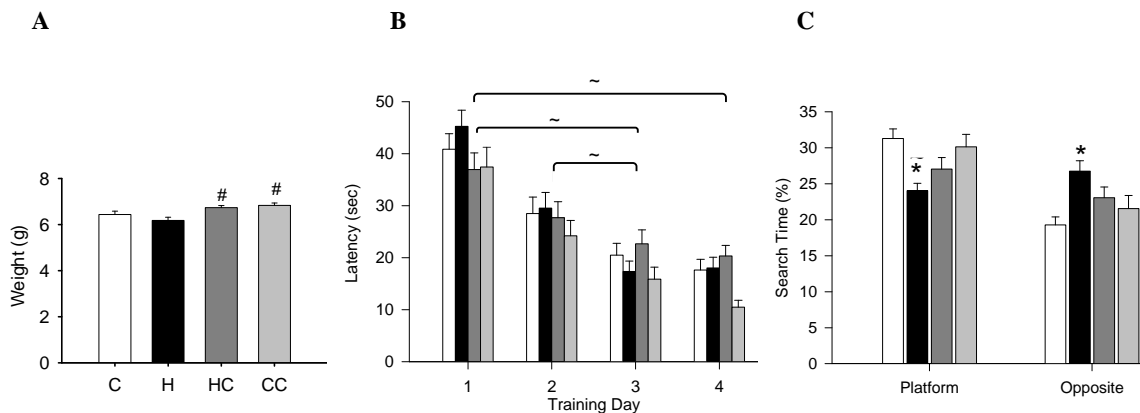
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**Introduction:** Prenatal hypoxia can impair memory consolidation in the chick<sup>1</sup>, and if combined with preterm delivery, can delay learning ability in lambs<sup>2</sup>. To date, it is unclear whether adverse conditions in pregnancy, such as prenatal hypoxia, can programme neurological impairments in adulthood. Moreover, the mechanisms underlying such impairments remain unknown. This study tested the hypothesis that the developmental programming of neurological disease by prenatal hypoxia is secondary to oxidative stress. We investigated in rats the effects of prenatal hypoxia on behaviour and cognitive function in adulthood, and determined whether vitamin C had any neuroprotective effects.

**Materials and Methods:** From days 6-21 of pregnancy, 44 female Wistar rats (n=5-6 per group) were divided into control (C: 21% O<sub>2</sub>) and hypoxic (H: 14% O<sub>2</sub>) pregnancies, with and without vitamin C (0.5g.100ml<sup>-1</sup> in drinking water). At birth, litters were culled to 8 pups, and offspring were weighed weekly until the completion of the study. At 3.5 months, Morris water maze and open field testing was performed to assess cognitive function and exploratory behaviour, respectively. To control for sex and within litter variation, only up to 2 male offspring from any one litter were studied (n=10-12 per group).

**Results:** Compared to controls, hypoxic pregnancies tended to reduce birth weight (Fig. 1A), and significantly increase catch-up growth from postnatal days 7-14 (fractional growth rate: H: +7±2%, P<0.05). Maternal treatment with vitamin C significantly improved birth weight in hypoxic pregnancies (P<0.05); fractional growth rates were unaltered (-0.4±2%). All rats were able to learn the position of the submerged platform in the water maze (decrease in latency, P<0.001, Fig. 1B). After four days of training, the platform was removed, and a probe test was performed. Relative to controls, hypoxic animals spent less time searching in the quadrant that had previously contained the submerged platform, and more time in the opposite quadrant (P<0.05, Fig. 1C), suggesting impaired memory retention. Maternal treatment with vitamin C significantly reduced thigmotactic (wall-hugging) behaviour (P<0.05, data not shown), and improved performance in the probe trial (P<0.05). There was no treatment effect on open field performance, as measured by the percentage of time spent in the centre and periphery of the arena, speed and path length.



**Figure 1.** Values are mean ± SEM for (A) birth weight (B) latency to find the submerged platform in the water maze over during training, and (C) search time in the target quadrant during the probe trial, in offspring of control (C, white), hypoxic (H, black), hypoxic + vitamin C (HC, dark grey) or control + vitamin C (CC, light grey) pregnancies. \*P<0.05 vs. control, #P<0.05 vs. hypoxic, ~P<0.05 vs. day 1 of training (One-way ANOVA or Two-way ANOVA + Tukey's test).

**Conclusions:** The data show that prenatal hypoxia can impair memory retention in adulthood. Maternal treatment with vitamin C improved performance in the Morris water maze following prenatal hypoxia, suggesting that oxidative stress could be a key link in the developmental programming of neurodegenerative disease.

Supported by the British Heart Foundation, The Royal Society and the BBSRC.

1. E.J.Camm *et al.*, *Reprod Fertil Dev.* 12: 165-172, 2000.

2. E.J.Camm *et al.*, *Dev Brain Res* 132: 141-150, 2001.

## A FETAL BRAIN INFLAMMATORY RESPONSE TO REPETITIVE UMBILICAL CORD OCCLUSIONS (UCO) WITH WORSENING ACIDOSIS IN THE OVINE FETUS NEAR TERM

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**Objective:** We have previously shown that the pro-inflammatory cytokine IL-1<sup>β</sup> is increased systemically in response to repetitive UCO leading to severe acidemia in the ovine fetus near term, likely due to altered placental perfusion with increased placental release. We sought to determine whether this inflammatory response is associated with local inflammation within the fetal brain, as measured by microglia (MG) and mast cell (MC) counts.

**Methods:** Near term fetal sheep (gestational age 125±2, term 145 d; control group n=6; UCO group n=10) were chronically prepared with arterial catheters and placement of an inflatable umbilical cord occluder. Following a baseline recording period, UCO group animals underwent a series of mild (1 min every 5 min), moderate (1 min every 3 min) and severe (1 min every 2 min) UCO each lasting 1h or until fetal arterial pH decreased to <7.0. Maternal and fetal blood samples were taken at selected time points for blood gases/pH and metabolites. Animals were euthanized at 24h of recovery with brain tissue processed for subsequent measurement of MG and MC cell counts per high power field (HPF) using brain immunohistochemistry and morphologic techniques in neocortex, white matter (WM), hippocampus, thalamus and choroid plexus.

**Results:** Repetitive UCO resulted in worsening acidosis over 3 to 4h eventuating in a severe degree of acidemia (fetal pH 7.36±0.01 (SEM) to 6.91±0.03; p<0.05). The MG cell count in WM and hippocampus was significantly increased in UCO vs control fetuses (31±5 vs 17±4; 36±12 vs 15±3 cells/HPF, respectively). Similarly, the MC cell count in the choroid plexus and thalamus was significantly increased in UCO vs control fetuses (1.0±0.2 vs 0.4±0.1; 0.3±0.1 vs 0 cells/HPF, respectively). While the degree of maximal fetal acidosis attained did not relate to the brain inflammatory findings, the total duration of repetitive UCO was highly correlated to WM / hippocampus MG cells/HPF for the UCO group animals, r=0.90, p<0.05.

**Conclusions:** MG and MC cell counts as measures of local inflammation within the brain are increased in response to repetitive UCO leading to severe acidemia in the ovine fetus near term, likely due to the associated increase in pro-inflammatory cytokines systemically which might then contribute as a co-factor to the increased risk for brain injury with severe acidemia at birth.

**EFFECT OF ACUTE HYPOXIA ON THE MOTOR ACTIVITY OF THE 10- AND 14-DAY CHICK EMBRYO.**

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**Introduction.**

Embryonic motility represents an important component of development. In vertebrates, active movement of the embryo is required for the correct development of the musculoskeletal and nervous systems. The disturbance of embryonic movements leads to severe malformations and functional disorders. It is known that embryonic motility in chick is periodic and consists of the activity phase (AP) and inactivity phase (IP). It changes during embryogenesis and depends on the environmental conditions. However, the effect of environmental influences on development, via the alteration of embryonic activity, is little investigated. The goal of our study was to analyze the effects of acute hypoxia on the embryonic motor activity in chick on incubation days 10 (D10) and D14.

**Materials and Methods.**

Chick eggs were incubated at 37.5°C. Eggs taken from the incubator on D10 or D14 were placed into a thermoregulated chamber with a continuous flow of warm atmospheric air and opened at the air cell side. A force transducer connected with the wing or leg of the embryo was used to record embryonic movements inside the egg. Force recording was synchronized with the video recording and stored digitally. The simple limb movements during IP were ignored in this study. During the experiment the recording was performed continuously in normoxia (30 min) then during acute hypoxia (10% O<sub>2</sub> for 10 min) and after that again in normoxia (30 min).

**Results.**

In normoxia, the mean duration of the AP was 36.4±2.2 sec on D10 and increased to 42.8±3.2 sec on D14; the IF was 66.2±3.2 sec on D10 and decreased to 28.7±1.3 sec on D14. Acute hypoxia did not affect the duration of the AP on both days studied. The mean duration of the IP showed the tendency to increase under hypoxia on D10 and significantly increased on D14. The analysis of the response dynamics to acute hypoxia on D14 has shown that IP started to increase since the first or second cycle, reached its maximum exceeded the control value by 4.5 times, and then recovered partly during the hypoxic exposure up to 150% of the control value. After the replacement of hypoxic mixture by the air the motor activity did not differ significantly from the control level on both ages studied.

**Conclusions.**

Response of the motor activity to hypoxia depended on the embryonic age. There were no significant changes both of the AP and IP in response to hypoxia on D10, but on D14, an inhibitory effect of hypoxia due to the increasing of the IP duration was found. The possible mechanism underlying the recovery of IP under hypoxia on D14 is discussed.

This work was supported by RFBR grant 08-04-01063.

## SESSION III: (Clinical/Translational)

Chair: Bill Parer

Monday September 28<sup>th</sup>

- 1:30pm – 1:45pm      Abstract 14:  
Fetal Tachyarrhythmias: The Comparison Between Cases with or without Intrauterine Treatment a Retrospective Data Analysis from Japanese Population  
K Ueda, T Ikeda, Y Maeno, N Inamura, M Kawatak, M Taketazu, M Nii, A Hagiwara, H Horigome, M Shozu, W Shimizu, S Yasukouchi, H Yoda, I Shiraishi, and H Sago
- 1:45pm – 2:00pm      Abstract 15:  
Fetal Heart Rate (FHR) Variability in Obstetric Cholestasis (OC).  
IAD Jayawardene, BR Hayes-Gill, PV Loughna, and F Broughton Pipkin.
- 2:00pm – 2:15pm      Abstract 16:  
Development of Human Photoreceptors  
Shinpei Watanabe, Hidenobu Ohta, Shizuko Akiyama, Takushi Hanita, Ai Obara, Kaori Imai, Yuichiro Miura, Ryuta Kitanishi, Tatsuya Watanabe, Masaki Satoh, Aya Tsujituka, Tadashi Matsuda, Shigeru Tsuchiya, Kunihiro Okamura, and Nobuo Yaegashi
- 2:15pm – 2:30pm      Abstract 17:  
Designing the Lighting Environments of the Neonatal Intensive Care Unit  
Shizuko Akiyama, Hidenobu Ohta, Shinpei Watanabe, 3 Takushi Hanita, Yuichiro Miura, Ryuta Kitanishi, Tadashi Matsuda, Tatsuya Watanabe, Kaori Imai, Yasuma Kumasaka, Junko Saitoh, Keiko Ueda, Shinji Katsuraki, Tomoaki Ikeda, Naoki Honma, Takahiro Moriya, Masayuki Iigo, Shigeru Tsuchiya, Kunihiro Okamura, and Nobuo Yaegashi
- 2:30pm – 2:45pm      Abstract 18:  
Early Postnatal Bronchoalveolar Lavage Fluid Growth Factor Patterns and Development of Bronchopulmonary Dysplasia  
Jasper V. Been, Anne Debeer, J. Freek van Iwaarden, Nico Kloosterboer, Valéria Lima Passos, Gunnar Naulaers, and Luc J. Zimmermann
- 2:45pm – 3:00pm      Abstract 19:  
Antenatal Steroids and Neonatal Outcome after Chorioamnionitis in Preterm Infants: Prospective Cohort Study and Meta-Analysis  
Jasper V Been, Pieter L Degraeuwe, Boris Kramer, Ingrid G Rours, René F Kornelisse, Tom A Schneider, Ronald R de Krijger, and Luc J Zimmermann

## **Fetal tachyarrhythmias: the Comparison between Cases with or without Intrauterine Treatment A Retrospective Data Analysis from Japanese Population**

Ueda K<sup>1</sup>, Ikeda T<sup>1</sup>, Maeno Y<sup>2</sup>, Inamura N<sup>3</sup>, Kawataki M<sup>4</sup>, Taketazu M<sup>4</sup>, Nii M<sup>6</sup>, Hagiwara A<sup>4</sup>, Horigome H<sup>7</sup>, Shozu M<sup>8</sup>, Shimizu W<sup>9</sup>, Yasukouchi S<sup>10</sup>, Yoda H<sup>11</sup>, Shiraishi I<sup>12</sup>, Sago H<sup>13</sup>

Department of Perinatology, National Cardiovascular Center (NCVC), Osaka, Japan<sup>1</sup>, Department of Pediatrics, Kurume University, Fukuoka, Japan<sup>2</sup>, Department of Pediatric Cardiology, Maternal and Child of Osaka, Osaka, Japan<sup>3</sup>, Department of Neonatology, Kanagawa Children's Hospital, Kanagawa, Japan<sup>4</sup>

Department of Pediatric Cardiology, Saitama Medical University, Saitama, Japan<sup>5</sup>, Department of Cardiology, Shizuoka Children's Hospital, Shizuoka, Japan<sup>6</sup>, Department of Pediatrics, Tsubaki University, Ibaraki Japan<sup>7</sup>, Department of Obstetrics and Gynecology, Chiba, Japan<sup>8</sup>, Department of Internal Medicine, NCVC, Osaka, Japan<sup>9</sup>, Department of Cardiology, Nagano Children's Hospital, Nagano, Japan<sup>10</sup>, Department of Neonatology, Japanese Red Cross Medical Center, Tokyo, Japan<sup>11</sup>, Department of Pediatric Cardiology, NCVC, Osaka, Japan<sup>12</sup>, Department of Perinatology, National Child Health Center, Tokyo, Japan<sup>13</sup>

**Introduction:** Fetal tachyarrhythmia (FT) is not a common fetal status which incidence is about 0.3-1/1000 of pregnancy. Although sustained FT occurs rarely, it results in heart failure, fetal hydrops (FH), and fetal/neonatal death. Transplacental anti-arrhythmic treatment for FT has been performed relatively often recently and known to be effective for rate control. However, the most essential data that how FT itself affects the natural history of FT and obstetrical outcome have been limited, because of lack of generalized vast majority of data.

**Purpose:** The aim of this study is to determine the impact of intrauterine treatment on natural history of FT, and to overview its efficacy and safety from the data of Japanese population.

**Methods:** The fetuses with sustained FT from 2004 to 2007 were registered. Data was accumulated by questioners from 750 perinatal care institutes in Japan. FTs including supraventricular tachycardia (SVT), atrial flutter and ventricular tachycardia were investigated for the fetal/neonatal diagnosis, presence of fetal hydrops (FH), associated cardiac anomalies, extra-cardiac complication, fetal/neonatal treatment, delivery mode, gestation of delivery, with or without fetal treatment the anti-arrhythmic agency of fetal treatment, the efficacy and adverse effect of intrauterine treatment and outcomes. In 2 groups obstetrical and neonatal prognoses were compared.

**Results:** 82 cases including 14 FH were analyzed. SVT was the most dominant fetal diagnosis (n=44). 6 cases were not accurately diagnosed in utero who were not referred to cardiologists, although fetal/neonatal cardiologists are involved in 70.6% of all cases. Fetal therapy was performed for 41 with a variety of agents, such as digoxin, flecainide and sotalol. The data showed high overall efficacy for FT (90.0%). Fetal treatment was effective even for the cases with FH (82.7%). When comparing to non-treated patients, treated cases showed significantly lower incidence of preterm birth (12.2% vs. 41.5%), cesarean-section (c/s) (29.3% vs. 70.7%, P<0.05) and neonatal arrhythmias. (48.8% vs. 78%, P<0.05)

**Discussion:** Intrauterine treatment has successful improved FT, even for the cases with HF. Also, this data showed that the main benefit of fetal treatment is to reduce premature birth, c/s and neonatal arrhythmias. In the present status, fetal treatment for FT was successfully performed in Japan. However, the difficulty of fetal diagnosis, especially in the cases without management of fetal/neonatal cardiologist was pointed out. Also no established guideline was suggested in this study.

**Conclusion:** This nation-wide retrospective data analysis clarified the beneficial effects of fetal therapy on the clinical course of FT. For further analysis, a multi-institutional prospective clinical trial should be planned.

## FETAL HEART RATE (FHR) VARIABILITY IN OBSTETRIC CHOLESTASIS (OC).

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**Introduction** Unexpected intrauterine fetal death in late gestation has been repeatedly reported in women with OC. This could be a consequence of altered cardiac conduction, presumably induced by increased bile acid concentration (BAC).

**Methods** The trans-abdominal fetal e.c.g. was acquired over ~12h using a Monica AN24 FHR monitor (Monica Healthcare Ltd, Nottingham)<sup>1</sup>. FHR was derived from the RR interval. The short term variability (STV) was calculated according to Dawes and Redman based on the 3.75second FHR averages. The root mean square of successive difference (RMSSD), a measure of true beat-to-beat variability, was also calculated. Data are summarised as mean  $\pm$  s.d. or median [IQR].

**Results and Discussion** Technically-satisfactory recordings of the fetal e.c.g. were obtained from 17 women with OC (pruritis with serum BAC  $>14\mu\text{mol/L}$  or ALT  $>100\text{U/L}$ ) and 17 women with uncomplicated pregnancies (NP). Median gestation ages at the time of recording were 30 – 38 weeks in OC and 29 – 38 weeks in NP ( $P>0.1$ ); median [IQR] BAC in OC was 38 [20 – 58] $\mu\text{mol/L}$  and ALT was 124 [75 – 159] $\text{U/L}$ .

A worse fetal outcome has been described in women with BAC  $\geq 40\mu\text{mol/L}$ <sup>2</sup>. Seven women had BAC ranging between 43 - 187 $\mu\text{mol/L}$  (median 67 $\mu\text{mol/L}$ ), while 10 OC were  $<40\mu\text{mol/L}$  (median 22.5 $\mu\text{mol/L}$ ). We therefore analysed the OC data in two groups, mild or moderate/severe. Neither FHR nor STV differed between groups (Table 1;  $P>0.5$ ,  $P>0.8$ ). However, the RMSSD was significantly higher in babies of women with moderate/severe OC than in either controls ( $P = 0.015$ ) or those with mild OC ( $P = 0.008$ ; ANOVA). Linear regression analysis revealed a highly-significant impact of individual BAC on RMSSD ( $P = 0.008$ ) and an inverse association with gestation age ( $P = 0.016$ ).

	Normal (n = 15)	Mild OC (n = 10)	Moderate/severe OC (n = 7)
FHR (bpm)	138.4 $\pm$ 6.7	137.8 $\pm$ 6.3	135.4 $\pm$ 5.2
STV (msec)	10.3 $\pm$ 1.9	10.7 $\pm$ 1.7	10.6 $\pm$ 2.1
RMSSD (msec)	10.6 $\pm$ 1.2	10.3 $\pm$ 0.9	12.2 $\pm$ 2.0

Table 1

We believe this to be the first time that a link has been observed between raised BAC and an alteration in an index of fetal vagal cardiac control, the RMSSD. The ability to record the beat-to-beat fetal e.c.g. transabdominally with the very small Monica AN24 monitor has allowed us to record for up to 16 hours overnight, with minimal maternal inconvenience, while she was completely relaxed.

**Comment** This will facilitate the identification of subtle changes in FHR variability and could reduce or abolish the need for alternate day use of antenatal cardiotocography in women with OC.

### References

1. Graatsma, E.M., *et al.* (2009) Fetal electrocardiography: feasibility of long-term fetal heart rate recordings. *BJOG: An International Journal of Obstetrics & Gynaecology* 116, 334-338
2. Glantz, A., *et al.* (2004) Intrahepatic cholestasis of pregnancy: Relationships between bile acid levels and fetal complication rates. *Hepatology* 40, 467-474

### Development of human photoreceptors

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The human retina contains three types of visual sensors (photoreceptors): rhodopsin, cone opsins, and melanopsin. KO studies have revealed that melanopsin in mice starts to function earlier than rhodopsin and cone opsins, and has also proved that melanopsin in mice, in addition to rhodopsin and cone opsins, is able to control their pupillary light reflex (PLR). We recently reported a preterm infant of 33 weeks' gestational age who did not show a PLR to the light wave length of 600 nm, which is outside the range of perceptibility of melanopsin but within the range of perceptibility of the other photoreceptors (Hanita et al., J Pediatr 2009 in press). The case report suggested the possibility that melanopsin is the only photoreceptor to start functioning within the early stages of development of human fetuses and preterm infants. We examined the hypothesis that melanopsin mainly contributes to the visual perception of immature infants and that other photoreceptors only develop well enough to function at later developmental stages in life.

Preterm infants of gestational ages between 34 and 36 weeks were selected for observation after obtaining parents' written consent to experimental procedures approved by the Tohoku University Ethics Board (#approval#2007-76). The subjects underwent pupil diameter measurements under different levels of illumination ranging from 0 to 16.0  $\mu\text{W}/\text{cm}^2$  of white light or a monochromatic light of 600 nm, both of which were generated by a 10 W EN20-1 bulb (Heine, Hersching, Germany) with or without a monochromatic light filter. The irises of each subject were videotaped for one minute in the dark prior to light exposure, and for 5 seconds during light exposure.

When tested with white light (7.57  $\mu\text{W}/\text{cm}^2$ ), preterm infants showed the same degree of high-amplitude constriction as adults, confirming the preterm infants sensitivity to light. In contrast, when tested with monochromatic light of 600nm, preterm infants showed no constriction at all, or that of a smaller degree than adults. This suggests that the photoreceptors of preterm infants are sensitive to a narrower range of wavelengths than adults' by being unable to detect the wavelength of 600nm(0-16  $\mu\text{W}/\text{cm}^2$ ), which is outside the detectable wavelength range of melanopsin, but within the range of rhodopsin or cone opsin.

Our data suggest a possible scenario: that melanopsin is the main photoreceptor functioning for visual perception at the early stages of human development and contributes to the detection of changes in environmental light radiance, and that rhodopsin starts to function sometime between 34 and 40 weeks' gestation and acts for image detection, and that finally cone opsins begin to function one month after birth and perform image and color detection.

### **Designing the lighting environments of the neonatal intensive care unit**

Shizuko Akiyama,<sup>1, 3</sup> Hidenobu Ohta,<sup>1, 2, 3</sup> Shinpei Watanabe,<sup>1, 3</sup> Takushi Hanita,<sup>1, 3</sup> Yuichiro Miura,<sup>1,3</sup> Ryuta Kitanishi,<sup>1,3</sup> Tadashi Matsuda,<sup>1,3</sup> Tatsuya Watanabe,<sup>4</sup> Kaori Imai,<sup>4</sup> Yasuma Kumasaka,<sup>4</sup> Junko Saitoh,<sup>4</sup> Keiko Ueda,<sup>5</sup> Shinji Katsuraki,<sup>5</sup> Tomoaki Ikeda,<sup>5</sup> Naoki Honma,<sup>6</sup> Takahiro Moriya,<sup>7</sup> Masayuki Iigo,<sup>8</sup> Shigeru Tsuchiya,<sup>3</sup> Kunihiro Okamura,<sup>1, 2</sup> and Nobuo Yaegashi,<sup>1, 2</sup>

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There are three types of lighting conditions that preterm infants may experience in NICUs: light-dark cycles, constant light, and constant dark. The lighting conditions of the NICU varies from country to country. In Japan, constant light is quite commonly used in NICUs while the US seems to favor constant-dark in their NICUs.

Research on optimum lighting environments for incubators has been carried out extensively through sleep research and chronobiology. Researchers have demonstrated through both clinical and molecular studies that

- 1) Preterm infants are likely to be light responsive already at around 25-28 weeks gestational age (Hao & Rivkees, PNAS 1999), and
- 2) A regular light-dark cycle is more appropriate for weight gain and sleep development of preterm infants than continuous light or continuous dark conditions (Man et al., BMJ 1986; Miller et al., Infant Behav Dev 1996; Brandon et al., J Pediatr 2002; Mirmiran & Ariano, Semin Perinatol 2001; Rivkees et al., Pediatrics 2004; Ohta et al., Nature Neurosci 2005; Ohta et al., Pediatr Res 2006).

In spite of growing evidence of the physiological benefits of nighttime exposure to darkness for infant development, many Japanese NICUs still prefer to maintain constant light in preparation for any possible emergencies arising in the incubators. To solve the dilemma between the negative effects of constant light on human development and the necessary lighting environments for emergency response in the NICU, we have developed a new device similar to a magic mirror, by which preterm infants can be shielded from exposure to light even in the constant light conditions of the NICU while simultaneously allowing medical care staff to visually monitor preterm infants without lighting problems. The effect of the device on preterm infants will be discussed in physiological parameters such as sleep development.

**EARLY POSTNATAL BRONCHOALVEOLAR LAVAGE FLUID GROWTH FACTOR PATTERNS AND DEVELOPMENT OF BRONCHOPULMONARY DYSPLASIA**

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**Introduction.** Chronic lung disease of prematurity (bronchopulmonary dysplasia; BPD) is characterised by an arrest in lung development. We hypothesised that early alterations in pulmonary expression of growth factors with key roles in normal lung development would precede the development of BPD.

**Methods.** Bronchoalveolar lavage fluid (BALF) was obtained from ventilated preterm infants on postnatal days 0, 1, 3, and 7 and analysed for total phospholipids, vascular endothelial growth factor (VEGF), platelet-derived growth factor-BB (PDGF-BB), transforming growth factor (TGF)  $\alpha$  and  $\beta$ 1, granulocyte macrophage colony stimulating factor (GM-CSF) and keratinocyte growth factor (KGF). Levels were transformed using the formula  $[\ln(\text{concentration}+1)]$  to account for both non-normality and non-detectable levels. Time-dependent expression patterns were compared between infants developing BPD (n=17) and BPD-free survivors (n=38), using a marginal model approach with adjustment for confounders. Predictive values of growth factor levels for BPD were computed using ROC curves.

**Results.** 121 BALF samples were analysed. BPD was associated with lower overall levels of VEGF ( $\beta(95\%CI)=-0.79(-1.37;-0.22)$ ;  $p=.007$ ), TGF- $\alpha$  ( $\beta(95\%CI)=-0.38(-0.73;-0.04)$ ;  $p=.03$ ) and latent TGF- $\beta$ 1 ( $\beta(95\%CI)=-1.13(-1.87;-0.39)$ ;  $p=.004$ ). Moreover, lower day 1 levels of PDGF-BB (mean difference(95%CI)=-1.01(-1.75;-0.26);  $p=.01$ ) and active TGF- $\beta$ 1 (mean difference(95%CI)=-0.95(-1.76;-0.15);  $p=.02$ ), and higher KGF at day 7 (mean difference(95% CI)=1.09(0.25;1.93);  $p=.02$ ) were associated with BPD development. VEGF levels on day 0 had the highest predictive value for BPD development (area under ROC-curve=0.87;  $p=.005$ ).

**Conclusions.** Substantial alterations in BALF growth factor levels are present in infants developing BPD. An early imbalance in pulmonary growth factors may contribute to the developmental arrest of the lung seen in BPD.

## ANTENATAL STEROIDS AND NEONATAL OUTCOME AFTER CHORIOAMNIONITIS IN PRETERM INFANTS: PROSPECTIVE COHORT STUDY AND META-ANALYSIS

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<sup>2</sup>Paediatrics, <sup>3</sup>Obstetrics, and <sup>4</sup>Pathology, Erasmus University Medical Centre, Rotterdam, Netherlands.

**Background.** Controversy exists regarding the risks and benefits of antenatal steroids in the setting of suspected intrauterine infection. Our aim was to investigate the association between antenatal steroids and neonatal outcome in a prospective cohort of preterm infants with chorioamnionitis and perform a meta-analysis on the subject.

**Methods.** In 301 preterm infants (gestational age  $\leq 32$  wks), relevant clinical data were documented and placental histology was performed. Patients were divided into groups based on the presence of either clinical chorioamnionitis (CC) or histological chorioamnionitis (HC). Within these groups, neonatal outcome parameters were compared between infants that had or had not received antenatal steroids.

In addition, MEDLINE and EMBASE were searched using the terms ‘chorioamnionitis’ and ‘steroids OR corticoids’, without constraints. Reference lists and citations of articles of interest were additionally screened. Studies were eligible for inclusion in the meta-analysis if they reported the incidence of selected neonatal outcome parameters in infants with chorioamnionitis according to antenatal steroid exposure: neonatal mortality, respiratory distress syndrome (RDS), bronchopulmonary dysplasia, patent ductus arteriosus (PDA), (severe) intraventricular haemorrhage (IVH), periventricular leucomalacia (PVL), necrotising enterocolitis and (early onset) sepsis. Analyses were separated for HC and CC. Meta-analysis (fixed and random effects models), study heterogeneity (Q statistic), and publication bias (Funnel plots and Egger’s regression test) were quantified using MIX 1.7 software. Study quality was assessed non-quantitatively.

**Results.** In the cohort, antenatal steroids in infants with HC (n=121) were associated with decreased RDS (51 vs. 72%; p=.04) and less need for surfactant (42 vs. 66%; p=.02). In infants with CC (n=93), antenatal steroids reduced the need for mechanical ventilation (76 vs. 100%; p=.049) and PDA surgery (4 vs. 23%; p=.009) and tended to decrease mortality (12 vs. 31%; p=.06).

In the meta-analysis six additional observational studies were included, all of similar study quality. No indications for publication bias or statistical heterogeneity were present. In infants with HC (N=5, n=1064) antenatal steroids were associated with reductions in neonatal mortality (OR[95%CI] = 0.45[0.30-0.68]; p<.0001), RDS (OR[95%CI] = 0.52[0.40-0.71]; p<.0001), PDA (OR[95%CI] = 0.56[0.37-0.85]; p=.007), IVH (OR[95%CI] = 0.35[0.19-0.66]; p=.001), and severe IVH (OR[95%CI] = 0.39[0.19-0.82]; p=.01). Four studies reported on infants with CC (n=397), showing reductions in severe IVH (OR[95%CI] = 0.28[0.09-0.87]; p=.03) and PVL (OR[95%CI] = 0.31[0.12-0.76]; p=.01) after antenatal steroids. No increase in adverse outcome was observed after antenatal steroids after either CC or HC.

**Conclusions.** In the current prospective cohort and meta-analysis of observational studies, antenatal steroids were associated with improved neonatal outcome in preterm infants with either suspected or documented intrauterine inflammation. Ideally, new RCTs with long-term follow up should address the safety and efficacy of antenatal steroids in the setting of suspected intrauterine infection.

**SESSION IV: (Pulmonary and Vascular Biology)**  
**Chair: Lubo Zhang**

Monday September 28<sup>th</sup>

- 3:30pm – 3:45pm      Abstract 20:  
Effects of Postnatal Steroid Administration on the Rat Model of Chronic Lung Disease  
Shouhei Konishi, Keiko Ueda, Kazutoshi Cho, Hisanori Minakami, and Yoshiyasu Kobayashi
- 3:45pm – 4:00pm      Abstract 21:  
Hemin Decreases Pulmonary Arterial Pressure in Hypertensive Newborn Lambs from the Andean Altiplano  
C Ebensperger, C Ulloa, RV Reyes, F Moraga, RT Rojas, P Silva, R Riquelme, J Ferrada, JT Parer, and AJ Llanos.
- 4:00pm - 4:15pm      Abstract 22:  
Antioxidant Treatment Prevents Peripheral Vascular Dysfunction Induced by Neonatal Glucocorticoids on Weanling and Adult Rats  
EA Herrera, MM Verkerk, and DA Giussani.
- 4:15pm – 4:30pm      Abstract 23:  
The Role of Calcium Activated Chloride Channels in Pulmonary Arterial Vasoconstriction is Influenced by Postnatal Maturity and Long-Term Hypoxic Stress  
S Vemulakonda, A Forrest, N Leblanc, JE Angermann, LD Longo, and SM Wilson
- 4:30pm – 4:45pm      Abstract 24:  
The Role of Nitric Oxide in Vasodilation Following Intrapleural OK-432 Administration in Preterm Fetal Sheep  
Laura Bennet, Rosalind V Cowie, Arlin B Blood, Ellen C Jensen, Peter R Stone, and Alistair J Gunn.
- 4:45pm – 5:00pm      Abstract 25:  
Does VEGF Mediate the Mitogenic Effects of Hypoxia in Large Arteries?  
William J Pearce, Stacy M. Butler, Jenna M. Abrassart, Rina M Dakanay, and James M. Williams

## EFFECTS OF POSTNATAL STEROID ADMINISTRATION ON THE RAT MODEL OF CHRONIC LUNG DISEASE

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### Introduction:

New bronchopulmonary dysplasia (new BPD) is a type of chronic lung disease (CLD) in neonate characterized by a fewer and larger alveoli, and is thought to be caused by an arrest of alveolarization and postnatal damage to immature lungs. Postnatal steroids are frequently used empirically for the treatment of severe CLD. However, effects of postnatal steroids on the postnatal development of lung structures have not been extensively studied. Accordingly, effects of postnatal dexamethasone (DXM) on the alveolarization of immature lungs are examined in this study using a rat model of new BPD which is previously developed by us.

### Methods:

We employed a rat model of new BPD previously described. In brief, 8-weeks WKAH/hkm rats were prepared for timed-pregnancy. On the 21<sup>st</sup> day of pregnancy, 1 µg of Lipopolysaccharide (LPS) was administered into each amniotic sac. The injection was performed by 30G needle immediately after the incision of abdomen under the general anesthesia. After 24 hours of this procedure, fetuses were extracted from uteri. Pups were resuscitated and nursed by foster rats. From the 1<sup>st</sup> to 4<sup>th</sup> days of age, DXM (with gradual decrease of the dose from 0.1 µg/g on 1 day, followed by 0.05 µg/g on 2 days, 0.025 µg/g on 3 days, 0.01 µg/g on 4 days) was administered subcutaneously to these pups. Same volume of saline was used as control of LPS or DXM. Pups were divided into 3 groups according to treatment regimen. 1) antenatal LPS+ postnatal DXM (DXM group, n=5), 2) antenatal LPS+ postnatal saline (LPS group, n=5), 3) antenatal saline+ postnatal saline (Control, n=5). At 14<sup>th</sup> days of age, the lungs were excised and fixed in 10% buffered formalin. Left anterior lungs were prepared as specimen and stained with hematoxylin and eosin (HE) which were examined by light microscopy; the alveolar surface density ( $S_v$ ), numerical density of alveoli ( $n_v$ ) and the average alveolar radius ( $r$ ) were calculated by morphometry. ANOVA was used for inter-group comparisons, and  $p$  values less than 0.05 were considered significant.

### Results:

There were no significant differences in the survival rate among the groups. Significantly fewer and larger alveoli were recognized in the LPS group and DXM group;  $S_v$  (/cm) was significantly reduced in the LPS group ( $460.5 \pm 37.4$ ) and DXM group ( $510.9 \pm 37.9$ ) than in the control group ( $706.8 \pm 11.5$ ), significantly smaller  $n_v$  (/mm<sup>3</sup>) was detected in the LPS group ( $242.9 \pm 99.4$ ) and DXM group ( $257.4 \pm 89.3$ ) than in the control group ( $1010.9 \pm 236.4$ ), and  $r$  (µm) was significantly longer in the LPS group ( $46.9 \pm 4.7$ ) and DXM group ( $44.2 \pm 5.8$ ) than in the control group ( $27.4 \pm 1.1$ ). There was no significant difference in  $S_v$ ,  $n_v$  and  $r$  between the LPS group and DXM group.

### Conclusion:

In the present experiment, postnatal administration of steroids did not induce a significant change in the pulmonary structure with respect to  $S_v$ ,  $n_v$  and  $r$  in the rats treated with antenatal LPS. These results suggest that postnatal steroids do not exert favorable effects on the clinical course of new BPD.

## HEMIN DECREASES PULMONARY ARTERIAL PRESSURE IN HYPERTENSIVE NEWBORN LAMBS FROM THE ANDEAN ALTIPLANO

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A crucial adaptation in the transition from fetus to newborn is the marked decrease in pulmonary arterial pressure (PAP) and vascular resistance. Pregnancy at high altitude in women and animals yields neonates with persistent pulmonary hypertension (1). Carbon monoxide is an endogenous gas produced by heme oxygenase that plays a role as antiproliferative and vasodilator agent (2,3). Hypothesis: Hemin, a heme oxygenase inductor, decreases the pulmonary hypertension in high altitude (HA) newborn (NB) lambs. Methods: Under ketamine anesthesia (5 mg/kg i.m.), 10 NB whose gestation and birth occurred in HA (Putre, 3,600m) were catheterized with a Swan-Ganz catheter placed into the pulmonary artery and polyvinyl catheters placed into femoral artery and vein, at 3 days of age. We measured PAP daily before and during the treatment with hemin for 10days (15 mg/Kg x day; Hemin Group n=5). We used 5 HA newborn lambs without hemin treatment as controls (Control Group). After 10 days of hemin treatment, we submitted the neonates to a superimposed episode of acute hypoxemia: 1h of normoxemia, 1h of hypoxemia and 1h of recovery. We measured blood gases, pulmonary arterial pressure, cardiac output (CO), heart rate (HR) and calculated pulmonary vascular resistance (PVR). In addition, we study *ex vivo* the vasoreactivity from small pulmonary artery, with wire myography. All the studies were performed at Putre Research Station (INCAS), 3,600m above the sea level. The Faculty of Medicine Ethics Committee of the University of Chile approved all experimental procedures. Results: The Hemin Group showed basally a decrease in the basal PAP compare to the Control Group ( $p < 0.05$ ). The PVR in the Hemin Group did not show any change during the 3 periods of the experimental protocol, whereas the Control Group showed a significant increase during the hypoxemic period ( $p < 0.05$ ). The CO, HR in the Hemin Group did not show statistical differences compared to the Control Group. The vasoreactivity studies showed a different vascular tone after ODQ administration, a soluble guanylate cyclase (sGC) inhibitor, in the Hemin Group ( $p < 0.05$ ). Conclusions: The hemin treatment decreased the pulmonary arterial pressure in high altitude pulmonary hypertensive newborn lambs and precluded the rise in PVR during the superimposed hypoxemic episode. This result may be due to an augment in the enzymatic activity of sGC and/or to lesser vascular remodeling in the pulmonary artery in the hemin treated neonates. Studies are in progress to investigate the latter. Supported by FONDECYT 1090355, 1080663-Chile.

### References:

- (1) Herrera EA et al. *Am. J. Physiol.* 292:R2234-R2240.2007.
- (2) Herrera EA et al. *Cardiovasc Res.* 77:197-201. 2008.
- (3) Ndisang JF et al. *J Hypertens.* 22:1057-1074. 2004

## ANTIOXIDANT TREATMENT PREVENTS PERIPHERAL VASCULAR DYSFUNCTION INDUCED BY NEONATAL GLUCOCORTICOIDS ON WEANLING AND ADULT RATS

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**Introduction** Despite the well established beneficial maturational effects of postnatal glucocorticoids, there is growing concern about unwanted side-effects (1), including alterations in vascular structure and function (2). The mechanisms underlying the detrimental effects of glucocorticoids are not fully understood, but oxidative stress may have a role (2,3). If true, treatment of glucocorticoids with antioxidants may preserve maturational, whilst preventing unwanted effects. This study tested the hypothesis that postnatal treatment with dexamethasone (Dex) using a clinical regimen programmes peripheral vascular dysfunction, and that these effects can be prevented by concomitant treatment with vitamins C and E.

**Materials and Methods.** Male Wistar pups received tapering I.P. injections of Dex (D; n=8; 0.5, 0.3, 0.1  $\mu\text{g}\cdot\text{g}^{-1}$ ) or Dex with vitamins C and E (DCE; n=8; 200  $\text{mg}\cdot\text{kg}^{-1}$ , and 100  $\text{mg}\cdot\text{kg}^{-1}$ , respectively) on postnatal days 1-3 (P1-3); vitamins were continued from P4-6. Controls received equal volumes (10  $\mu\text{l}\cdot\text{g}^{-1}$ ) of vehicle (C; n=8) from P1-6. A fourth group received vitamins alone (CCE, n=8). A set of rats were euthanized at weaning (P21) and another at adulthood (P100). Femoral arterial rings were dissected and mounted on a wire myograph and concentration-response curves to vasoconstrictors (potassium,  $\text{K}^+$  and phenylephrine, PE), and vasodilators (methacholine, MetCh and sodium nitroprusside, SNP) were generated.

**Results and Discussion.** D markedly decreased survival (80.6%) compared to C (100%). Co-administration of vitamins C and E significantly improved pup survival (96.4%,  $P<0.05$ ).

Relative to C, the femoral maximal contraction to  $\text{K}^+$  was significantly reduced both at weaning and adulthood (Fig.1). D pups showed increased maximal contraction (% $\text{K}_{\text{max}}$ ,  $84.7\pm 4.8\%$  vs  $67.5\pm 5.8\%$ ,  $P<0.05$ ) and sensitivity ( $\text{pD}_2$ ,  $7.56\pm 0.21$  vs  $6.42\pm 0.18$ ,  $P<0.05$ ) to PE. Notably, all the above effects were prevented by the combined treatment of glucocorticoids with vitamins C and E.

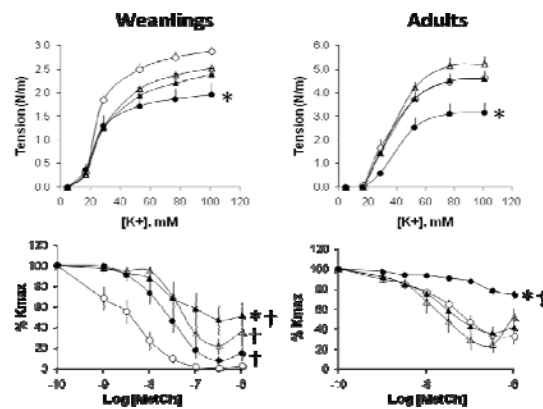
Relative to C, all treatment groups showed significantly reduced vascular sensitivity to MetCh, but no alteration to the SNP response, at weaning. Only DCE pups showed a decreased maximal relaxant response to MetCh. By adulthood, D markedly decreased the dilator response to MetCh ( $\text{PD}_2$  and % $\text{K}_{\text{max}}$ , Fig. 1) and to SNP relative to controls ( $\text{PD}_2$ ,  $6.99\pm 0.05$  vs  $7.14\pm 0.09$ ,  $P<0.05$ ). DCE completely prevented the effects of Dex on femoral vascular relaxation to MetCh (Fig.1) and SNP ( $\text{PD}_2$ ,  $7.28\pm 0.06$ ) in adults.

**Conclusions.** Postnatal treatment of rats with glucocorticoids using a human clinical dosing regimen has detrimental effects on survival and peripheral vascular function at weaning and adulthood. Concomitant treatment of dexamethasone with antioxidant vitamins prevents the programming effects of glucocorticoids on vascular dysfunction.

Supported by the British Heart Foundation, BBSRC and The Royal Society.

### References

- Halliday *et al.* Cochrane Database Syst Rev. (1):CD001146, 2009.
- Iuchi *et al.* *Circ Res*, 92: 81-87, 2003.
- Rajashree, Puvanakrishnan. *Mol Cell Biochem*, 181: 77-85, 1998.



**Figure 1.** Concentration-response curves to  $\text{K}^+$  and MetCh for weanlings (P21) and adult (P100) rats. Mean  $\pm$  SEM for control (C,  $\circ$ , n=8), Dex (D,  $\bullet$ , n=8), Dex + Vit.CE (DCE,  $\blacktriangle$ , n=8) and Vit.CE alone treated neonates (CCE,  $\triangle$ , n=8). Significant differences ( $P<0.05$ ): \* Max response, † sensitivity vs Ctrl (ANOVA + Tukey test).

## THE ROLE OF CALCIUM ACTIVATED CHLORIDE CHANNELS IN PULMONARY ARTERIAL VASOCONSTRICTION IS INFLUENCED BY POSTNATAL MATURITY AND LONG-TERM HYPOXIC STRESS

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**Introduction:** Pulmonary artery contractility is dependent on membrane depolarization and resultant increases in cytosolic calcium. Agonists, such as serotonin, often induce pulmonary arterial contractility through membrane depolarization, but the specific mechanism(s) involved in the depolarization process remain controversial. Experimental evidence over the past decade indicates that calcium-activated chloride channels (ClCa) play a pivotal role in the membrane depolarization response, which then modulates extracellular calcium entry (3, 5). Our previous investigations show that Ca<sup>2+</sup> signaling due to 5-HT is blunted in fetal pulmonary arterial myocytes (1) and that the role of L-type Ca<sup>2+</sup> channels (Ca<sub>L</sub>) to 5-HT-elicited contractility is reduced by long-term hypoxia (LTH) in fetal and adult sheep. Thus, we wished to determine the extent to which the role for ClCa changes in parallel with Ca<sub>L</sub>. We therefore tested three hypotheses: 1) that maturation leads to an increased role for ClCa whilst LTH depresses the role of ClCa during 5-HT mediated pulmonary arterial contractility; 2) that ClCa-mediated contractility is dependent on functional Ca<sub>L</sub>; and 3) that TMEM16A, a molecular candidate for ClCa (2, 4), is expressed in sheep pulmonary arteries. **Materials and Methods:** These hypotheses were assessed by performing wire-myography, Ca<sup>2+</sup> imaging, and RT-PCR analysis on isolated pulmonary arteries from fetal, newborn and adult sheep. Fetal and adult sheep were either normoxic or hypoxic, where hypoxia was induced by housing pregnant and non-pregnant ewes at 3,801 m for ~ 110 days. **Results and Discussion:** Pulmonary arterial constriction to 10 μM 5-HT in adult was substantially inhibited by the ClCa blocker niflumic acid (100 μM), and LTH diminished this effect. Niflumic acid modestly reduced tension in 5-HT contracted arteries from normoxic and hypoxic fetuses. The influence of niflumic acid on contractility was restrained in adult when Ca<sub>L</sub> was inhibited with 10 μM nifedipine. The imaging studies suggest that ClCa inhibition has complex effects on cytosolic Ca<sup>2+</sup>, with possible changes in the number of responsive cells, their firing rate and magnitude of the Ca<sup>2+</sup> rise. RT-PCR analysis show that TMEM16A is expressed in sheep pulmonary arteries. **Conclusion:** These studies provide the first report indicating postnatal maturity increases ClCa functionality in the pulmonary vasculature while hypoxic stress may blunt this effect. The finding that TMEM16A is expressed in pulmonary arterial segments suggests this gene product may be responsible for the effects of niflumic acid on these arteries and influenced by development and hypoxic stress. (Supported by NIH P01HD031226 and R01HD03807 to LDL, and R01 HL075477 and NCRR P20 RR15581 to NL).

### References:

1. Goyal et al., *AJP-LCMP* 295: L905-914, 2008.
2. Hartzell et al, *J Physiol*, 587:2127-2139, 2009.
3. Leblanc et al, *Canadian J of Physiol and Pharm* 83: 541-556, 2005.
4. Schroeder et al, *Cell* 134: 1019-1029, 2008.
5. Yuan *AJP-Renal* 272: L959-L968, 1997.

**The role of nitric oxide in vasodilation following intrapleural OK-432 administration in preterm fetal sheep**

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**Background:** Altered blood flow is observed after exposure to infection and inflammatory agents, particularly during the first 12-24 hours. Cerebral hyperperfusion is frequently observed at all ages, and is commonly attributed to increased release of vasodilators such as nitric oxide (NO). We have recently observed in the preterm fetus that exposure to a single dose of killed gram positive streptococci (OK-432) results in both acute and chronic changes in blood flow. The aim of this study was to evaluate whether changes in NO activity contribute to these changes.

**Methods:** Preterm fetal sheep at 0.7 gestation were given 0.1mg OK-432 (n=5), or normal saline (n=6) via an intrapleural catheter. Fetal mean arterial pressure (MAP), carotid and femoral blood flow (CaBF and FBF respectively) were monitored *in utero* from 1 day before until 7 days after injection. Blood samples were taken before and after injection to measure nitrite concentrations as an index of nitric oxide synthase activity.

**Results:** Acutely OK-432 was associated with initial decrease in CaBF (between 3-6 hours after injection ( $p<0.05$ ), followed by an increase peaking at 18 hours ( $P<0.05$ ). Similar trends were seen in FBF. These changes in blood flow were mediated by vascular resistance changes not altered MAP. In the days following injection, there was a gradual, but persistent increase in both CaBF and FBF ( $P<0.05$ ), mediated by decreased vascular resistance. MAP was lower than control group values from day 4 after injection ( $P<0.05$ ). There was no change in nitrite concentrations over time or between groups.

**Conclusions:** The lack of change in nitrite concentrations following OK-432 injection suggests that altered NO synthase activity does not mediate the acute or chronic changes in blood flow. However, we cannot rule out increased vascular sensitivity to NO (hypereactivity), and other vasodilators may play a role. Alternatively, the fetus may have developed impaired reactivity to vasoconstrictors (hyporeactivity). How long this vasodilatation lasts, and the implications for perinatal and indeed adult well-being remain to be determined.

**DOES VEGF MEDIATE THE MITOGENIC EFFECTS OF HYPOXIA IN LARGE ARTERIES?**

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Chronic hypoxia induces in many tissues numerous metabolic and structural adaptations initiated in large part by Hypoxia Inducible Factor 1 $\alpha$  (HIF-1 $\alpha$ ) (6). In turn, HIF-1 $\alpha$  activates the transcription of multiple genes, including Vascular Endothelial Growth Factor (VEGF) and erythropoietin, that exert potent mitogenic effects on vascular tissues (2, 4). Whereas the angiogenic effects of VEGF on microvascular endothelium are well established, the mitogenic effects of VEGF on the endothelium, smooth muscle, and perivascular nerves of muscular arteries remains uncertain. Recent evidence suggests that VEGF may serve autocrine functions in larger arteries (5), and may promote growth of perivascular autonomic nerves (3), which in turn can potently influence vascular differentiation (1). In light of this evidence, the present study examines the hypothesis that *VEGF mediates the mitogenic effects of chronic hypoxia in large arteries through effects on: 1) large artery endothelium; 2) medial smooth muscle; or 3) perivascular nerves*. Pregnant and non-pregnant sheep were maintained at either sea level or at an altitude of 3820 m for the final 110 days of gestation. Some fetal sheep underwent a superior cervical ganglionectomy 14 days prior to term to denervate the cerebrovasculature, and then were returned to the womb and allowed to develop to term. At term, carotid and middle cerebral arteries were harvested from fetal and adult sheep, then placed in organ culture. Following 48 hours of organ culture in the absence of serum and the presence or absence of physiological concentrations (3 ng/ml) of recombinant human VEGF-A<sub>165</sub>, artery contractility was characterized using active and passive stress-strain relations, after which expression of multiple phenotypic markers ( $\alpha$ -actin, myosin light chain kinase, regulatory light chain, SM1 myosin, SM2 myosin, and protein kinase G-I) were assayed using Western immunoblotting and fluorescent immunohistochemistry with confocal visualization. In endothelium-denuded arteries, organ culture in VEGF induced multiple highly significant hypoxia-dependent changes in artery stiffness, contractility, and marker protein expression. Organ culture with VEGF also induced multiple significant changes in endothelium-intact arteries, and the pattern of changes observed was markedly different than in endothelium-denuded arteries. Finally, sympathetic denervation significantly altered both the pre-culture and post-culture characteristics of the arteries in a highly age-dependent and hypoxia-dependent manner. Together, these data strongly support the general hypothesis that VEGF can mediate the mitogenic effects of chronic hypoxia on large arteries through separate direct effects on large artery endothelium, medial smooth muscle, and perivascular adrenergic nerves. Thus, VEGF appears to be an important mediator of both angiogenic and non-angiogenic vascular effects of chronic hypoxia.

**Literature Cited**

1. Damon DH. Sympathetic innervation promotes vascular smooth muscle differentiation. *Am J Physiol Heart Circ Physiol* 288: H2785-2791, 2005.
2. Hoeben A, Landuyt B, Highley MS, Wildiers H, Van Oosterom AT, and De Bruijn EA. Vascular endothelial growth factor and angiogenesis. *Pharmacol Rev* 56: 549-580, 2004.
3. Marko SB and Damon DH. VEGF promotes vascular sympathetic innervation. *Am J Physiol Heart Circ Physiol* 294: H2646-2652, 2008.
4. Nakano M, Satoh K, Fukumoto Y, Ito Y, Kagaya Y, Ishii N, Sugamura K, and Shimokawa H. Important role of erythropoietin receptor to promote VEGF expression and angiogenesis in peripheral ischemia in mice. *Circ Res* 100: 662-669, 2007.
5. Osada-Oka M, Ikeda T, Imaoka S, Akiba S, and Sato T. VEGF-enhanced proliferation under hypoxia by an autocrine mechanism in human vascular smooth muscle cells.
6. Semenza GL, Shimoda LA, and Prabhakar NR. Regulation of gene expression by HIF-1. *Novartis Found Symp* 272: 2-8; discussion 8-14, 33-16, 2006.

## SESSION V: (Perinatal Programming)

Chair: Laura Bennett

Tuesday September 29<sup>th</sup>

- 8:00am – 8:15am      Abstract 26:  
Effects of a “Western” Diet on Maternal Metabolism And Fetal Development in Rats  
C. Gray, ME Symonds, S Gardiner and DS Gardner
- 8:15am – 8:30am      Abstract 27:  
Episodic Ethanol Exposure has Multiple Effects on the Fetus  
R Harding, K Kenna, V Stokes, H Parkington, M Tare, S Gray, A Bocking, J Brien, F Sozo, S Rees, and D Walker
- 8:30am – 8:45am      Abstract 28:  
Effects of Moderate Prenatal Ethanol Exposure on Lactation, Mammary Gland Development and Pup Growth  
Megan E Probyn, Emma-Kate Lock, Chelsea G Stewart, Mary E Wlodek, John Bertram, and Karen M Moritz
- 8:45am – 9:00am      Abstract 29:  
Impact of Fetal Exposure to Maternal Melatonin on Amount and Functionality of Brown Adipose Tissue (BAT) in the Newborn.  
M Mondaca, H Reynolds, C Torres-Farfan, N Mendez, L Abarzua-Catalan, FJ Valenzuela, R Ebensperger, AJ Llanos, GJ Valenzuela, and M Seron-Ferre
- 9:00am – 9:15am      Abstract 30:  
Vitamins C and E Ameliorate the Programming of Cardiac Dysfunction in Adult Rats Induced by Neonatal Dexamethasone Treatment  
Y. Niu, EA Herrera, RD Evans, and DA Giussani
- 9:15am – 9:30am      Abstract 31:  
Early Life Undernutrition in Sheep Induces Sex- and Tissue-Specific Effects on Factors Mediating Insulin Sensitivity and Lipid Handling  
KR Poore, A Warlow, A Brewin, JK Cleal, D Noakes, MA Hanson and LR Green
- 9:30am – 9:45am      Abstract 32:  
Antenatal Protein Malnutrition in the Mouse: Epigenetic Changes and Developmental Origin of Hypertension in Adult  
Ravi Goyal, Arthur Lietzke, Dipali Goyal, and Lawrence D. Longo
- 9:45am – 10:00am      Abstract 33:  
Enhanced Adipose Tissue Desaturation Activity Promotes Programmed Obese Phenotype in Intrauterine Growth Restricted Newborns  
JK Yee, MG Ross, WN Paul Lee, and M Desai

**EFFECTS OF A “WESTERN” DIET ON MATERNAL METABOLISM AND FETAL DEVELOPMENT IN RATS.** C. Gray<sup>1</sup>, M.E. Symonds<sup>2</sup>, S. Gardiner<sup>3</sup> and D.S. Gardner<sup>1</sup>. 1. School of Veterinary Medicine and Science, Sutton Bonington. 2. School of Human Development. 3. School of Biosciences, Queens Medical Centre, University of Nottingham.

**Introduction:** Maternal diet can influence the offspring’s susceptibility to cardiovascular disease (CVD)<sup>1</sup>. Dietary excess, particularly of fructose and salt, is characteristic of a typical ‘Westernised’ diet consumed by pregnant women. We therefore aimed to determine the effects of moderately high maternal fructose and/or salt intake on maternal metabolism, fetal development and cardiovascular health of adult offspring. **Methods:** 32 virgin Sprague Dawley rats were randomly divided into 4 dietary groups; 1) control diet (CD, n=8) fed purified chow and tap water, 2) Salt diet (SD, n=8) fed purified chow + 4% NaCl, 3) Fructose diet (FD, n=8) fed purified chow and 10% fructose in tap water and 4) Fructose & Salt diet (FSD, n=8), fed 4% NaCl purified chow with 10% fructose in the drinking water. Animals were fed ad libitum for 28 days prior to conception and to day 20 of gestation, whereupon they were euthanised. Blood samples were taken 14 days prior to conception and at day 20 of gestation for analysis of protein, fat and carbohydrate metabolism using an autoanalyser (RX-IMOLA, Randox). Cardiovascular data are currently being obtained by radiotelemetry (Datasciences Int). The data were analysed by a 2x2 repeated measures factorial design using in Genstat v11 (VSNi. UK) and are presented as predicted means. **Results:** Prior to feeding the experimental diets, there was no significant difference between the body weights of dams (220±10g). CD, SD and FD animals gained similar amounts of weight during the first 28 days of feeding, but by day 10 FSD animals began to gain significantly (P<0.001) less weight when compared to all other groups. With fructose consumption, (FD and FSD) food intake was significantly (P<0.001) reduced (by 10-20g/day) and fluid intake significantly (P<0.001) increased (by 23±5 and 42±5ml/day, respectively). As a result, total energy intake was not different between groups. Fructose intake increased plasma glucose, triglyceride, NEFA and uric acid concentration (P<0.001) with only glucose remaining higher in FD, relative to other groups, during pregnancy (P=0.029). Groups consuming increased salt showed a significant increase in kidney and heart weights (P<0.001). Fructose feeding specifically increased maternal liver weight by 20-25% (P<0.001) e.g. FD (15.22±0.32g), FSD (14.64±0.32g) vs. CD (12.17±0.37g). In addition, a significant redistribution of adipose tissue in the dams consuming fructose was observed; relative to CD, the gonadal depot in FD was reduced (FD, 4.91±0.51g vs. CD, 7.15±0.59) but perirenal depot increased (FD, 2.96±0.11g vs. CD, 1.98±0.13). There were few effects of the maternal diets on fetal metabolite profiles or on body or placental weights in male and female offspring but litter size was reduced in FSD (an average of 7 vs. 14 pups). **Discussion:** The data indicate that increased maternal intake of fructose and of salt can have marked effects on maternal metabolism and on her organ size; however the fetus appears largely protected from these effects at this stage. We are currently following up the offspring from these pregnancies and hope to present some of these preliminary results at FNPS.

#### References

1. McMillen C, Robinson JS. Developmental Origins of the Metabolic Syndrome: Prediction, Plasticity, and Programming, *Physiol Rev* 85: 571-633, 2005.

## EPISODIC ETHANOL EXPOSURE HAS MULTIPLE EFFECTS ON THE FETUS

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J Brien<sup>3</sup>, F Sozo<sup>1</sup>, S Rees<sup>4</sup>, D Walker<sup>1</sup>

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**Background:** During human pregnancy, episodic exposure of the fetus to ethanol (EtOH) is not uncommon. Although the characteristics of the fetal alcohol syndrome are well known, the effects of lesser degrees of exposure on organ development are not well understood. As fetal EtOH exposure may be a potent, but little recognised cause of developmental programming, we have used a sheep model to explore the effects of prenatal EtOH exposure on organ development.

**Aims:** Our aims were to determine the effects of repeated EtOH exposure during the last trimester equivalent on the development of major organs.

**Methods:** Pregnant ewes with implanted jugular vein catheters were infused with either saline or EtOH (0.75g/kg) for one hour daily from 95 days of gestational age (DGA) until 133 DGA (term ~147 DGA). At 125 DGA, the animals underwent surgery for the insertion of a fetal arterial catheter for blood sampling and arterial pressure/heart rate recording, and an amniotic catheter. At necropsy (134 DGA) we collected tissues for analysis.

**Results:** Maternal and fetal plasma EtOH concentrations reached maximal values of ~0.11g/dL at 1 hour after the infusion onset; by 8h, maternal and fetal levels were essentially zero. Each daily EtOH exposure resulted in transient mild hypoglycemia in the ewe and fetus as well as maternal acidemia and delayed fetal hypoxemia. There was no effect of EtOH exposure on fetal body or organ weights, and no gross anomalies were seen. In the fetal lungs, EtOH exposure led to increased collagen mRNA expression and collagen deposition, and reductions in mRNA expression of surfactant proteins A and B to ~33% of control levels. The mRNA expression of the pro-inflammatory cytokines IL-1 $\beta$  and IL-8 was reduced by ~90% compared with control levels (Sozo et al, 2009). In the fetal kidneys, there was an 11% reduction in nephron endowment following EtOH exposure (Gray et al, 2008). In fetal arteries from several vascular beds, EtOH led to altered smooth muscle reactivity and endothelial function, and a profound increase in arterial stiffness. In the fetal brain, we found that EtOH led to thinning of the corpus callosum and a decreased number of microglia in white matter.

**Conclusions:** Daily exposure of the fetus to EtOH during the later stages of gestation can impact upon the development of multiple organs. Mechanisms may include fetal hypoxemia, hypoglycemia and oxidative stress. If the alterations in organ structure persist after birth, they could impair health in later life. On-going studies are examining the postnatal effects of fetal EtOH exposure.

### References

Gray SP et al (2008) Repeated ethanol exposure during late gestation decreases nephron endowment in fetal sheep. *Am J Physiol: Regul, Integr Comp* 295; R568-574.

Sozo F et al (2009) Repeated ethanol exposure during late gestation alters the maturation and innate immune status of the ovine fetal lung. *Am J Physiol: Lung Cell Mol Physiol*. 296; L510-8.

**EFFECTS OF MODERATE PRENATAL ETHANOL EXPOSURE ON LACTATION, MAMMARY GLAND DEVELOPMENT AND PUP GROWTH**

Megan E Probyn<sup>1</sup>, Emma-Kate Lock<sup>1</sup>, Chelsea G Stewart<sup>1</sup>, Mary E Wlodek<sup>2</sup>, John Bertram<sup>3</sup>, Karen M Moritz<sup>1</sup>

<sup>1</sup>University of Queensland, Australia; <sup>2</sup>The University of Melbourne, Australia; <sup>3</sup>Monash University, Australia

**Introduction:** It is well known that high levels of alcohol consumption during pregnancy can lead to preterm birth and low birth weight. Low birth weight has also been identified as a risk factor for the development of adult onset diseases. While some pregnant women continue to consume low levels of alcohol during pregnancy it is unknown what the effects of low to moderate alcohol consumption will be on the fetus or offspring. In addition, it is unknown whether any dose of alcohol consumption alters the structure and development of the maternal mammary gland. Using a rodent model of ethanol (EtOH) exposure (15% calories derived from EtOH), the **aim** of this study was to determine if low to moderate EtOH consumption throughout pregnancy alters: 1) early postnatal growth of the offspring, 2) pup milk intake in the early pre-weaning period, and 3) maternal mammary gland structure and development.

**Materials and Methods:** 60 time mated virgin Sprague-Dawley rats were fed an *ab-libitum* approximately isocaloric liquid diet  $\pm$  6% EtOH throughout the entirety of pregnancy. Daily measurements of maternal diet consumption and weight gain were recorded. A subset of dams were killed at embryonic day (E) 20 (n=11 EtOH and n=9 Control) or postnatal day (PN) 1 (n=9 EtOH and n=8 Control) and the mammary glands collected for gene expression and histological analysis. The remaining dams (n=10 EtOH and n=13 Control) were allowed to spontaneously litter down and pup body weight was recorded daily. At PN3, 6, 9, 12 and 15 pup milk intake was determined via the weigh-suckle-weigh (WSW) technique. Male and female offspring were analysed separately.

**Results:** EtOH-exposed pups were not growth restricted at PN1 when they were first weighed (Control males  $7.05 \pm 0.30$ g, EtOH males  $6.41 \pm 0.24$ g, Control females  $6.69 \pm 0.27$ g, EtOH females  $6.30 \pm 0.18$ g). At PN6, but not at other ages, EtOH-exposed pups consumed less milk than their control counterparts (weight gain during WSW: Control males  $0.31 \pm 0.04$ g, EtOH males  $0.15 \pm 0.03$ g (t-test  $P=0.009$ ), Control females  $0.32 \pm 0.07$ g, EtOH females  $0.14 \pm 0.03$ g (t-test  $P=0.048$ )). EtOH-exposed pups appear to be growing slower than Control pups after this time (EtOH-exposed males 3% and 10% smaller at PN6 and PN28, respectively; EtOH-exposed females - 1% and 7% smaller at PN6 and PN28, respectively). Mammary glands were of similar weight between groups at both E20 and PN1. However, preliminary data suggests that the maternal mammary glands of EtOH fed dams (n=7) have less alveoli and blood vessels and more adipose tissue than that of control dams (n=6) at E20. These studies are ongoing.

**Conclusions/Summary:** Low to moderate prenatal EtOH exposure may also induce postnatal growth restriction with a potential mechanism being attributed to decreased maternal mammary gland development and milk production.

**Discussion:** Our results demonstrate the first rodent model of EtOH exposure that has suggested a role for adverse mammary gland development and milk production in the programming of postnatal growth restriction of EtOH exposed offspring. Our preliminary data derived from a low to moderate EtOH exposure indicates that the mammary gland should be considered in other high dose EtOH exposure models.

**IMPACT OF FETAL EXPOSURE TO MATERNAL MELATONIN ON AMOUNT AND FUNCTIONALITY OF BROWN ADIPOSE TISSUE (BAT) IN THE NEWBORN.**

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In human and sheep newborns, brown adipose tissue (BAT), accrued during pregnancy is used for thermogenesis. During cold exposure, triglycerides (TAG) stored in BAT cells are hydrolyzed to glycerol and fatty acids. BAT mitochondria possess UCP1, protein that uncouples oxidative phosphorylation of fatty acids to produce heat. In the fetus, BAT lipolysis is inhibited by factors secreted or transported by the placenta. Maternal melatonin crosses unaltered the placenta and is absent in the newborn. Given that melatonin inhibits norepinephrine-induced lipolysis in fetal sheep BAT (Torres-Farfan et al, J Physiol. 2008, 586:4017-4027) we propose that maternal melatonin helps to accrue BAT to be used in the newborn thermogenic response. *Purpose:* To assess the role of maternal melatonin deprivation and replacement during gestation a) on in vivo thermogenic and lipolytic (glycerol) response to cold exposure of the newborn lamb and b) on newborn perirenal BAT weight, morphology, TAG content and glycerol response to norepinephrine. *Methods:* Maternal melatonin was suppressed by exposing 9 pregnant sheep to constant light from 63% until delivery (term 145 days). Four sheep received daily oral melatonin replacement (12 mg). Controls were 5 newborns from mothers maintained in 12h light:12h dark. At 2 days of age, newborns were instrumented (vascular catheters, a Swan-Ganz and a thermistor in perirenal BAT). At 4-6 days of age, the newborns were exposed for 1 hour at 25C, followed by 1 hour at 4C and one hour at 25C. Oxygen consumption and glycerol production were measured at 15 min intervals and perirenal temperature was measured continuously. Upon completion of the experiment, lambs were euthanized and perirenal BAT was dissected. Pieces of BAT were preserved in Trizol for UCP1 mRNA measurement, criopreserved for histology and portions of BAT were used fresh in culture. BAT explants (~25 mg) were pre-incubated in triplicate for 6-h at 37°C in culture medium followed by 12 hours in 2 ml medium alone (basal) or containing 0.1 µM of norepinephrine. Glycerol production was measured in the supernatants and TAG content in the explants. *Results:* The absence of maternal melatonin during gestation had no effect on the newborn thermogenic response to cold measured as oxygen consumption, but depressed the glycerol response to cold. The amount of BAT weight/kg body was decreased, showing smaller size (area) unilocular cells with an increased UCP1 expression. In vitro, BAT explants showed decreased basal TAG content, increased basal lipolysis and blunted response to norepinephrine. These effects were reversed in BAT from newborns whose mothers received melatonin during pregnancy.

*Conclusion:* Exposure to maternal melatonin during gestation is important in determining amount and functionality of BAT in the newborn. Support: FONDECYT 1060766-1090381

## VITAMINS C AND E AMELIORATE THE PROGRAMMING OF CARDIAC

### DYSFUNCTION IN ADULT RATS INDUCED BY NEONATAL DEXAMETHASONE TREATMENT

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**Introduction:** Despite its significant role in preventing and treating chronic lung diseases in premature infants, adverse effects of dexamethasone (Dex) on cardiovascular development have made the clinical application of the medicine controversial. Some short-term adverse side effects of postnatal Dex on cardiovascular function have been reported and these are thought to be transient. However, whether postnatal Dex programmes longer-term adverse effects on cardiovascular function remains to be elucidated. Moreover, the mechanism underlying these unwanted side-effects of glucocorticoids are not fully understood, but oxidative stress may have an important role. In this study, we used adult rats to investigate: 1) the effects of neonatal Dex administration on isolated cardiac mechanical function, and 2) if postnatal co-administration of vitamins C and E can protect against Dex effects on the adult heart.

**Materials and Methods:** Male Wistar pups received i.p. injections of a clinical regimen of dexamethasone (Dex; n = 8; 0.5, 0.3, 0.1  $\mu\text{g}\cdot\text{g}^{-1}$ ) or Dex with vitamins C and E (DCE; n = 8; 200  $\text{mg}\cdot\text{kg}^{-1}$  and 100  $\text{mg}\cdot\text{kg}^{-1}$ , respectively) on postnatal days 1-3 (P1-3); vitamins were continued from P4-6. Controls received equal volumes (10  $\mu\text{l}\cdot\text{g}^{-1}$ ) of saline (ctrl; n = 8) from P1-6. Rats were euthanized at P100 and isolated hearts were perfused under working mode and then transferred to the “Langendorff” mode. Cardiac mechanical function was evaluated and the responses of hearts to different workloads were determined.

#### Results and Discussion:

Dex-treated animals had a lower heart weight relative to body weight ( $0.00274 \pm 1.08\text{E-}4$  vs  $0.00333 \pm 1.64\text{E-}4$ ) but cardiac output and hydraulic work were both maintained ( $54.3 \pm 6.8$  vs  $48.8 \pm 4.2$   $\text{ml}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$  heart weight;  $3648.7 \pm 237.5$  vs  $3053.3 \pm 343.0$   $\text{mmHg}\cdot\text{ml}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$  heart weight, respectively), suggesting that cardiac systolic function was unaffected. However, left ventricular end diastolic function (LVEDP) of hearts

from Dex-treated rats was increased relative to controls, suggesting that cardiac diastolic function was impaired. Interestingly, concomitant treatment with vitamins C and E eliminated the effects of Dex (Fig. 1A). The response of mean aortic pressure (MAP) to increased afterload in Dex-treated animals doubled compared to control, and treatment of vitamins C and E reversed this enhanced response. In addition, in Dex-treated offspring, the cardiac output (CO) responses to a decrease in preload decreased significantly. Again, concomitant treatment with vitamins C and E restored the cardiac responses back to normal (Fig. 1B).

**Conclusions:** Postnatal treatment of rats with Dex using a human clinical regimen programmes cardiac dysfunction in adulthood, an effect that can be prevented by antioxidant vitamins.

Supported by the British Heart Foundation, the BBSRC and The Royal Society

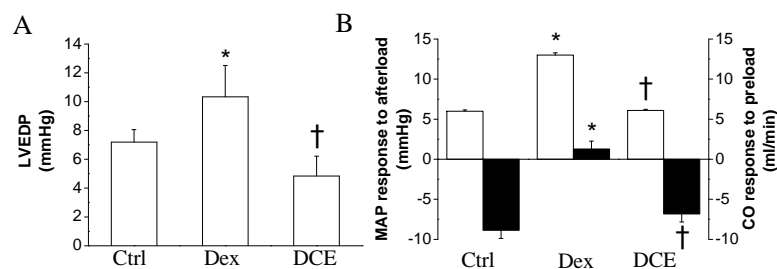


Fig 1. Mean  $\pm$  SEM. A, LVEDP; B, responses of MAP to an increase in afterload (open bars), and of CO to a decrease in preload (close bars). Significant differences ( $P < 0.05$ ), \* vs control, † vs Dex (ANOVA+Tukey test).

**EARLY LIFE UNDERNUTRITION IN SHEEP INDUCES SEX- AND TISSUE-SPECIFIC EFFECTS ON FACTORS MEDIATING INSULIN SENSITIVITY AND LIPID HANDLING**

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**Introduction:** Improved insulin sensitivity may be one mechanism to accelerate growth following a period of growth retardation. We have demonstrated previously that poor growth in early postnatal life, induced by nutrient restriction, enhances glucose tolerance/insulin sensitivity in female but not male adult sheep <sup>(1)</sup>. However, this may have a subsequent negative impact on glucose handling if it led to inappropriate adipose tissue deposition <sup>(1)</sup>. To understand the mechanisms involved in increased glucose tolerance following postnatal undernutrition, this study examined components of the insulin signalling pathway (insulin receptor (IR) and GLUT4) in skeletal muscle and adipose tissue in our model of adult sheep exposed to nutrient restriction in early gestation and/or early postnatal life. Mediators of lipid handling (lipoprotein lipase, LPL) and adipocyte differentiation (PPAR- $\gamma$ ) were also examined in adipose tissue.

**Methods:** Ewes received either 100% (C, n=36) or 50% nutritional requirements (U, n=34) from 1-31 days gestation and 100% thereafter. Male and female offspring from single and twin pregnancies were then fed either *ad libitum* (CC, n=22; UC, n=13) or to reduce body weight to 85% of target from 12-25 weeks postnatal age (CU, n=14; UU, n=21) and *ad libitum* thereafter. At post mortem at age 2.5 years, skeletal muscle and peri-renal adipose tissue were collected in liquid nitrogen. Real-time RT PCR was used to measure mRNA expression for IR and GLUT4 in both tissues and for LPL and PPAR- $\gamma$  in adipose tissue. Gene expression was normalised to mean  $\beta$ -actin and GAPDH mRNA expression. Data were analysed by ANOVA and linear regression.

**Results:** In females, postnatal undernutrition increased IR (CU and UU:  $1.54 \pm 0.13$  vs. CC and UC:  $1.13 \pm 0.14$ ;  $P < 0.05$ ) and GLUT4 (CU and UU:  $1.17 \pm 0.06$  vs. CC and UC:  $0.94 \pm 0.09$ ;  $P < 0.01$ ) mRNA expression in muscle, regardless of the prenatal nutrient environment. This was not observed in adipose tissue. Reduced growth rate from 12-25 weeks of age was directly associated with increased IR and GLUT4 ( $R^2 = -0.17$  and  $-0.22$ , respectively,  $P < 0.05$ ) in muscle from females. In males, IR and GLUT4 mRNA expression was unaffected by early life nutrition however LPL mRNA expression was increased in those exposed to undernutrition in early gestation, regardless of the postnatal nutrient environment (UC and UU:  $1.20 \pm 0.13$  vs. CC and CU,  $0.84 \pm 0.07$ ). PPAR- $\gamma$  mRNA expression was not different between the groups.

**Conclusions:** This study has shown sex- and tissue-specific differences in factors that regulate insulin signalling and lipid handling following early life undernutrition. The increase in IR and GLUT4 mRNA expression in skeletal muscle in adult females exposed to postnatal undernutrition suggest that the improved glucose tolerance in these animals is due to increased insulin sensitivity in muscle but not adipose tissue. However, there was no evidence that this effect was also associated with factors that may predispose to inappropriate fat deposition. Rather, an increase in adipose tissue LPL mRNA expression in prenatally undernourished adult males may lead to an inappropriate balance between circulating and stored lipids, although no effects on fatness were observed in these animals <sup>(1)</sup>.

(1). Poore *et al.* (2007) **Am J Physiol Endocrinol Metab** 292(1): E32-9.

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## ANTENATAL PROTEIN MALNUTRITION IN THE MOUSE: EPIGENETIC CHANGES AND DEVELOPMENTAL ORIGIN OF HYPERTENSION IN ADULT

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**Introduction:** In laboratory animals, several studies have demonstrated that maternal protein restriction can result in the development of hypertension in the offspring. Surprisingly, no study reports development of hypertension as a consequence of maternal low protein diet (MLPD) in mice. Being a valuable tool for genetic/mechanistic analysis, in FVB/NJ mice we tested the hypothesis that maternal low protein diet during gestation leads to developmental programming of hypertension, and related disorders such as intra-uterine growth retardation, type II diabetes mellitus, and obesity. We also determined the extent to which MLPD leads to epigenetic changes in transcriptional regulators (promoter DNA methylation) and post-transcriptional regulators (microRNA-mediated regulation) of the angiotensin converting enzyme (ACE) in brain. **Methods:** We administered isocaloric, normal (control), 50% (moderate MLPD) and 33% (severe MLPD) diet, starting one week before mating and continuing until delivery of the pups. From 4 weeks following delivery, by use of a non-invasive tail-cuff method, we measured blood pressure in the offspring. We also isolated DNA, mRNA and proteins from the mice brain at fetal, 3 weeks and 33 weeks of age. We performed Realtime PCR, Western Immunoblot, miRNA assays, Bisulfite modification of DNA, cloning and sequencing to study various aspects of genetic and epigenetic alterations in the brain. Antisense microRNA and microRNA mimickers were transfected in mouse endothelioma cell lines to study post-transcriptional regulation of observed epigenetic alterations and their functional effects. To determine serum insulin and leptin levels ELISA kits were used, Onetouch glucose strips and system was used to measure blood glucose levels. We used computerised tomography scanning to measure adipose tissue volume in live mice from MLPD and the control groups. **Results and Discussion:** Birth weight was significantly reduced in both males and females from moderate and severe MLPD group, as compared to the control. Rapid catch-up group was observed in these low birth weight offspring from both MLPD groups, as compared to the controls. Both moderate and severe MLPD lead to significant increase in blood pressure in male and female mice, as compared to the controls. Increased blood glucose was observed in moderate MLPD female group with no change in insulin levels. However, increased adipose tissue volume and leptin levels were observed only in severe MLPD group females, as compared to controls. In both fetal and adult MLPD brain, we observed an increase in ACE mRNA levels. However, in the fetus increased ACE mRNA was not associated with increased protein levels. MicroRNA mmu-mir-27a increased significantly in fetus from moderate MLPD group, which can lead to decrease in ACE protein translation. We also observed hypomethylation of DNA promoter of ACE, which can increase mRNA transcription. **Conclusion:** Overall our results indicate that MLPD can lead to epigenetic changes in brain ACE with associated hypertension in adult offspring. We speculate that these findings may have profound implications for the health and well being of adult individuals who were subjected to protein deprivation *in-utero*.

## ENHANCED ADIPOSE TISSUE DESATURATION ACTIVITY PROMOTES PROGRAMMED OBESE PHENOTYPE IN INTRAUTERINE GROWTH RESTRICTED NEWBORNS

J.K. Yee<sup>2</sup>, M.G. Ross<sup>1</sup>, W.N. Paul Lee<sup>2</sup>, M. Desai<sup>1</sup>

<sup>1</sup>Dept. of Ob/Gyn, <sup>2</sup>Dept. of Pediatrics, LABioMed at Harbor-UCLA Med. Ctr.

**Introduction:** Intrauterine growth restriction (IUGR) leads to increased risk of adult obesity and lipid abnormalities. Targets for prevention and treatment of obesity include stearyl-CoA desaturase enzyme 1 (SCD1) which is expressed in metabolically active organs. In adipose tissue, liver and muscle, SCD1 converts stearate to oleate (C18:0 to C18:1) and palmitate to palmitoleate (C16:0 to C16:1). Further, oleate modulates central appetite suppression, which is impaired in IUGR offspring. Maternal undernutrition during rat pregnancy results in IUGR newborns. When allowed rapid catch-up growth, IUGR offspring develop hypertrophic adipocytes at 3 weeks of age prior to the development of hypertriglyceridemia and overt obesity, implicating adipose tissue as a primary source of these abnormalities. We thus hypothesized that upregulated SCD1 in IUGR offspring leads to increased desaturation indices in adipose tissue prior to onset of obesity. The desaturation indices (ratios of oleate/stearate and palmitoleate/palmitate) which represent a measure of SCD1 activity was studied in 3 week male IUGR and Control offspring.

**Methods:** Control dams received ad libitum food from day 10 to 21 of gestation, and study dams were 50% food-restricted to produce IUGR pups. All pups were nursed by Control dams and male offspring were studied at 3 weeks of age. Adipose tissue (non-visceral subcutaneous and visceral retroperitoneal), liver, muscle, and plasma samples were saponified, fatty acids extracted, and GC/MS performed. Desaturation indices were determined for the oleate to stearate ratio and palmitoleate to palmitate ratio from the relative intensities of gas chromatogram peaks. Values are means±SE.

**Results:** IUGR males exhibited increased SCD1 activity in *adipose tissue*, evidenced by significantly increased oleate/stearate desaturation index in subcutaneous fat ( $3.5\pm 0.1$  vs.  $3.2\pm 0.1$ ,  $p<0.05$ ) and retroperitoneal fat ( $3.2\pm 0.1$  vs.  $2.8\pm 0.1$ ,  $p<0.05$ ). Similarly, palmitoleate/palmitate desaturation index was increased in both fat depots of IUGR as compared to Controls (subcutaneous:  $0.06\pm 0.01$  vs.  $0.04\pm 0.01$ ,  $p<0.05$ ; retroperitoneal -  $0.04\pm 0.01$  vs.  $0.03\pm 0.01$ ,  $p<0.05$ ). In contrast to adipose tissue, IUGR males showed significantly decreased *liver* oleate/stearate desaturation index ( $0.19\pm 0.01$  vs.  $0.25\pm 0.03$ ,  $P<0.01$ ). *Muscle* and *plasma* desaturation indices were comparable to those of the Controls and the palmitoleate/palmitate desaturation index was undetectable in liver, muscle and plasma. Lastly, the overall liver stearate to palmitate ratio was significantly increased in IUGR males as compared to Controls ( $1.51\pm 0.02$  vs.  $1.38\pm 0.01$ ,  $p<0.01$ ).

**Conclusions:** In IUGR male offspring, the reduced liver desaturation index together with elevated stearate to palmitate ratio indicates augmented stearate accumulation, either from increased production or decreased desaturation. Additionally, the higher desaturation index in IUGR adipocytes reflects increased propensity toward fat accrual owing to the ability of adipose tissue to store more oleate than stearate. These findings suggest that programmed changes in adipose tissue may be the major contributory factor leading to adult obesity in IUGR offspring. As these finding occur prior to the development of obesity, preventative approaches may be applicable during early postnatal life.

**SESSION VI: (Neurobiology and Endocrinology)**  
**Chair: Graham Jenkin**

Tuesday, September 29<sup>th</sup>

- 10:30am – 10:45am      Abstract 34:  
Behavioural Effects of Altering Pregnane Steroid Concentrations in The Brain of Normoxic and Asphyxiated Fetal Sheep  
Tamara Yawno, Jon Hirst, Edwin Yan, and David Walker
- 10:45am – 11:00am      Abstract 35:  
How Each Neuron of the Mammillary Body Forms Projections to Thalamic and Midbrain Nuclei Using Collaterals of its Axon  
IG Makarenko., and EV Alpeeva
- 11:00am – 11:15am      Abstract 36:  
Maturation of the Generation and Control of Sympathetic Nerve Activity  
LC Booth, SC Malpas,, CJ Barrett, SJ Guild, AJ Gunn, and L Bennet
- 11:15am – 11:30am      Abstract 37:  
Developmental Programming of Dysfunctional Hypothalamic Neural Stem Cells in Leptin Deficient, Low Birth Weight Newborns  
M Desai, T Li, and MG Ross
- 11:30am-11:45am      Abstract 38:  
Interaction of NMDA-Glutamatergic Neurotransmission and Local Prostaglandin Biosynthesis in the Stimulation of the Fetal Hypothalamus-Pituitary-Adrenal Axis.  
C Wood, I Sampaio, and N Knutson

**BEHAVIOURAL EFFECTS OF ALTERING PREGNANE STEROID  
CONCENTRATIONS IN THE BRAIN OF NORMOXIC AND ASPHYXIATED  
FETAL SHEEP**

Tamara Yawno<sup>1,2</sup>, Jon Hirst<sup>3</sup>, Edwin Yan<sup>4</sup>, David Walker<sup>1</sup>

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**Introduction:** Pregnane steroids such as allopregnanolone (AP) have been thought to be involved in maintaining fetal sleep, while their withdrawal at birth might allow arousal of the neonate to occur. The synthesis and release of AP, a potent GABAergic agonist that suppresses central nervous system (CNS) activity, has been shown to increase rapidly following prenatal asphyxia in sheep.

**Aims:** The aims of this study were to determine the effect of suppressing AP production using finasteride, a 5 $\alpha$ -reductase type-2 (5 $\alpha$ R-2) inhibitor, on CNS activity of fetuses under normal conditions and following transient asphyxia caused by occluding the umbilical cord (UCO) for 5 mins. We determined if the effects of finasteride were specific to suppressing 5 $\alpha$ R-2 activity by co-infusing the synthetic allopregnanolone analogue, alfaxalone.

**Methods:** Pregnant ewes underwent surgery at ~125 days gestational age for insertion of fetal catheters, electrodes for measurement of the electrocorticogram (ECoG) and nuchal electromyogram (EMG), and for placement of an inflatable occluder around the umbilical cord. At ~130 days GA fetuses received infusion of either finasteride (40 mg/kg/h; n = 5), alfaxalone (5 mg/kg/h; n = 5) or finasteride + alfaxalone (n = 5). Further groups (4 groups of fetuses; n = 5 in each group) were subjected to 5 min UCO at 30 mins after the start of each infusion regime.

**Results:** In non-UCO fetuses, finasteride treatment alone significantly increased the incidence of low voltage (LV) ECoG (at 4 – 24 h post-infusion) and significantly increased the incidence of behavioural arousal (at 14 – 16 h post-infusion), effects that were reduced by co-infusion of alfaxalone with finasteride. In UCO fetuses, finasteride treatment caused a significant decrease in the incidence of both high voltage (HV) and LV ECoG and a significantly prolonged the presence of the sub-low voltage ECoG compared to UCO in vehicle-treated fetuses; again, these effects were prevented by co-infusion of alfaxalone. Alfaxalone also reduced the number of seizure-like spikes produced by UCO.

**Discussion:** These results confirm that neurosteroids significantly modulate CNS activity in the normoxic, late gestation fetus, and have effects that modify and limit the effects of asphyxia on the fetal brain, consistent with their ability to limit the cell death caused by such stress.

## HOW EACH NEURON OF THE MAMMILLARY BODY FORMS PROJECTIONS TO THALAMIC AND MIDBRAIN NUCLEI USING COLLATERALS OF ITS AXON.

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**Introduction.** Axonal projections originating from the mammillary bodies (MB) represent important pathways that are essential for spatial information processing. Two compact efferent tracts emerging from MB were described in adult vertebrates (mammillotegmental and mammillothalamic tracts). Specific feature of these fiber bundles innervating tegmental nuclei, pons and anterior thalamic nuclei is that they are composed of axonal collaterals of the MB neurons. The aim of this study was to describe the consequence of the formation of main axonal projection systems of mammillary bodies during perinatal ontogenesis.

**Materials and Methods.** Carbocyanine dye tracing was used on the rats with dated pregnancy. The day of insemination and birth were considered as E0 and P0 respectively. Fetuses from E14,5 and postnatal rats P0-P60 were fixed by perfusion with 4% paraformaldehyde and consecutive immersion. After brain dissection from the skull crystals of DiI were inserted into the mammillary bodies and tegmental region and stored in the same fixative. Labeled nerve cells and fibers were revealed on serial 80-100 µm vibratome sections using fluorescent and confocal microscopy.

**Results.** DiI insertion into MB reveal mammillotegmental tract in the midbrain on E14,5 without any signs of collateralization. Its axons start innervate tegmental nuclei on E18 using fine branches oriented dorsally from the main bundle. Later on P2 all three tegmental nuclei are filled of terminal network of MB axons. Labeled neurons in these nuclei do not send axons in mammillotegmental and form mammillary peduncle from late prenatal stages. First collaterals of the mammillotegmental axons growing in rostradorsal direction became visible from E17. During next 2 days they form compact mammillothalamic tract, which fibers grow simultaneously and have growth cones at their ends. On E20-21 they enter the ventral region of the anterior thalamic nuclei and form first terminal arborizations there. MB projections to the anteromedial and anteroventral thalamic nuclei are unilateral. On P2 second order collaterals are formed at the level of anterodorsal thalamus and cross the midline. These axons belong to the neurons of the lateral mammillary nucleus and innervate bilaterally anterodorsal thalamic nuclei. On P6-P10 innervation of anterior thalamic nuclei is fully developed.

**Conclusion.** Development of MB axons and formation of their collaterals takes place on the precise stages of perinatal development.

This work was supported by RFBR grant 07-04-00798.

### References:

- Alpeeva E.V., Makarenko I.G. Perinatal development of the mammillo-tegmental connections in rats. *Russian Journal of Developmental Biology*, 2007, 38 (2) March-Apr.: 58–65. Translated from *Ontogenez*, 2007, 38 (2):86–93.
- Alpeeva E.V., Makarenko I.G. Perinatal development of the mammillothalamic tract and innervation of the anterior thalamic nuclei. *Brain Research*, 2009, 1248, 1-13.
- Makarenko, I.G., DiI tracing is a useful tool for studies of the hypothalamic connections during perinatal development. In *Neural Pathways Research*. Chapter II. F.L. Pichler, ed. Nova Sci. Publishers, New-York, 2008, pp. 31-71.

**Maturation of the generation and control of sympathetic nerve activity**

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Department of Physiology, The University of Auckland, New Zealand.

**Introduction:** Direct recordings of sympathetic nerve activity (SNA) have two main properties. The first is the presence of co-ordinated bursts of SNA, indicative of many nerve fibres firing at approximately the same time. The second is entrainment of the discharges to the cardiac cycle, which is widely believed to be dependent on input from baroreceptors to a network of cell groups within the central nervous system. Although this patterning is used as a 'gold standard' for the identification of successful nerve recordings, the maturation of these basic features of SNA have not previously been investigated.

**Materials and Methods:** Using a telemetry-based nerve amplifier, renal SNA was recorded in preterm ( $99 \pm 1$  days gestation; term 147 days) and near-term ( $119 \pm 0$  days gestation) fetal sheep, without the confounding effects of anaesthesia or paralysis, and contrasted to renal SNA recorded in adult sheep.

**Results:** Recordings across all three age groups showed a classic bursting pattern in SNA and co-ordination of bursts with the cardiac cycle. However, the delay between diastole and the next peak in SNA, used as a measure of the baroreceptor entrainment of SNA to the cardiac cycle, was most pronounced in the preterm fetus ( $319 \pm 1$  ms), shorter in the near-term fetus ( $250 \pm 13$  ms) and shortest in the adult sheep ( $174 \pm 38$  ms).

**Conclusions:** Although entrainment of SNA to the cardiac cycle suggests that the intricate circuitry within the CNS is present even in the immature fetus, the present study suggests rapid further maturation of this key regulatory system in late gestation.

## DEVELOPMENTAL PROGRAMMING OF DYSFUNCTIONAL HYPOTHALAMIC NEURAL STEM CELLS IN LEPTIN DEFICIENT, LOW BIRTH WEIGHT NEWBORNS

M. Desai, T. Li, M.G. Ross

*Dept. of Ob/Gyn, LABioMed at Harbor-UCLA Med. Ctr.*

**Introduction:** Appetite regulatory circuits in the hypothalamus develop in utero to assure neonatal food intake, and the set-points for appetite regulation are programmed during the gestational and lactational newborn periods. Low birth weight (LBW) offspring have a programmed dysfunction in hypothalamic neuronal development with reduced anorexigenic neural pathways and dysregulation of central signaling of orexigenic and anorexigenic neuropeptides. Programmed hyperphagia and obesity in LBW offspring results, in part, from impaired anorexigenic mechanisms. Although leptin and insulin serve as hypothalamic modulators of appetite/satiety in the adult, they have critical neurotrophic properties during fetal life, including development of hypothalamic appetite pathways. As LBW offspring have decreased cord blood leptin and insulin levels, we hypothesized that reduced neurotrophic stimulation during critical periods may alter the development of appetite pathways. We utilized neural stem cells (NSC) to investigate the growth and differentiation of hypothalamic neuronal cells, in response to leptin and insulin. We hypothesized that LBW-associated leptin-deficiency (LD) causes impaired neuronal NSC proliferation and differentiation.

**Methods:** Control dams received ad libitum food, whereas study dams were 50% food-restricted from pregnancy day 10 to 21 to produce LBW-LD newborns. At day 1 of age, hypothalamus was dissected and cultured in complete medium (CM) containing growth factors and heparin. At day 8-9 of culture, NSC were digested and seeded in CM or differentiating medium (DM; without growth factors and heparin) for basal studies. For studies of neurotrophic proliferation responses, NSC cultured in CM were treated with leptin (10, 20, 40 ng/ml) or insulin (10, 20, 40 µg/ml) every 48h for 8 days and proliferation rate measured by MTT assay. For differentiation responses, NSC cultured in DM were treated with leptin or insulin (as above) and cell differentiation quantified by expression (Western Blot) of neuronal (NeuN, Tuj1) or astrocyte (GFAP) markers.

**Results:** The *basal proliferation index* of NSC was significantly reduced in LD newborns (15%). Although LD and Control NSC responded to leptin and insulin with dose-dependent increments in proliferation, LD NSC displayed reduced *proliferation* at all doses as compared to Controls (50-60%). Further, LD had reduced *basal differentiation* to both neuronal (Tuj1, 22%) and astrocyte (GFAP, 42%) cell lines, as compared to Controls. In response to leptin, both LD and Controls showed dose-dependent increments in differentiation though at all times, the LD exhibited reduced neuronal (~34%) and astrocyte (~29%) differentiation as compared to Controls. In response to insulin, both LD and Controls showed dose-dependent increment only in neuronal differentiation. Once again, LD exhibited reduced neuronal (~32%) and astrocyte (~40%) differentiation as compared to Controls.

**Conclusions:** Low birth weight, leptin-deficient newborns have programmed dysfunctional hypothalamic neuronal stem cells, evident by reduced basal and stimulated proliferation and neuronal/astrocyte differentiation. These results indicate that impaired NSC proliferation and differentiation is the likely etiology for reduced anorexigenic neural pathways in LBW offspring, and contribute to the resulting hyperphagia and obesity.

Interaction of NMDA-Glutamatergic Neurotransmission and Local Prostaglandin Biosynthesis in the Stimulation of the Fetal Hypothalamus-Pituitary-Adrenal Axis. Charles E. Wood, Isabela Sampaio, and Nathan Knutson. Department of Physiology and Functional Genomics and Department of Pediatrics, University of Florida College of Medicine, Gainesville, FL.

Prostaglandins, generated within the fetal brain, are integral components of the mechanism controlling the fetal hypothalamus-pituitary-adrenal axis. Previous studies in this laboratory have demonstrated that COX-2 inhibition reduces the fetal HPA response to cerebral hypoperfusion, blocks the preparturient rise in fetal plasma ACTH concentration, and delays parturition. At the same time, we have discovered that blockade of NMDA receptors in the fetal brain eliminate the fetal ACTH response to cerebral hypoperfusion. The present study was designed to test the hypothesis that COX-2 action and the downstream effect of HPA axis stimulation is itself stimulated by NMDA-glutamatergic neurotransmission. Chronically catheterized late-gestation fetal sheep were subjected to intravenous injection of NMDA (1 mg, iv; n=8). All responded with increases in fetal plasma ACTH and cortisol concentrations. Pretreatment with intravenous injection of resveratrol (100 mg, iv; n=5), a specific inhibitor of COX-1, did not alter the magnitude of the HPA axis response to the NMDA. Pretreatment with nimesulide (10 mg, iv; n=6), a specific inhibitor of COX-2, produced a non-significant reduction in the HPA response to NMDA. To further explore this interaction, we injected NMDA in 6 chronically catheterized fetal sheep that were chronically infused with nimesulide (n=6) at a rate of 1 mg/day into the lateral cerebral ventricle for 5-7 days. Only half of the nimesulide-treated fetuses responded to the NMDA with increased HPA axis activity, and overall the summary response was not statistically significant. Finally, we tested whether the HPA response to prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) is mediated by NMDA receptors. Seven chronically catheterized late-gestation fetal sheep were subjected to injection of 100 ng PGE<sub>2</sub>, a treatment that significantly increased fetal plasma ACTH and cortisol concentrations. Pretreatment with ketamine (10 mg, iv), a treatment that we have shown to block the fetal ACTH response to cerebral hypoperfusion, did not alter the magnitude of the ACTH or cortisol response to the PGE<sub>2</sub>. We conclude that generation of prostanoids via the action of COX-2 in the fetal brain augments the fetal HPA axis response to NMDA-mediated glutamatergic stimulation. These *in vivo* studies do not reveal the neuroanatomical substrate for the NMDA-COX-2 interaction. Recent studies in our laboratory in which we have immunostained glutamatergic neurons with anti-*vglut2* and then probed for COX-2 immunoreactivity with anti-COX-2 have revealed that neurons in the ventrolateral medulla express both *vglut2* and COX-2. Other regions potentially involved in the reflex control of HPA axis activity are currently being studied.

**POSTER DISCUSSION SESSION**  
**Chairs: Charles Wood and Maureen Keller-Wood**

Tuesday September 29<sup>th</sup>  
8:00 pm-10:00 pm

- Poster 1: Abstract 39  
Programmed Enhanced Adipogenesis Contributes to Adult Obesity in Growth Restricted Offspring: Evidence From Ex Vivo Adipose Cell Culture  
M. Desai, TR Magee, G Han, MG Ross.
- Poster 2: Abstract 40  
Small Increases in Fetal Cortisol Increase Expression of ENaC $\alpha$  in Fetal Lung without Changing Expression of Other ENaC Subunits or Aquaporin.  
Xiaodi Feng and Maureen Keller-Wood
- Poster 3: Abstract 41  
Repeated Exposure To TNF- $\alpha$  In An *In Vitro* Ovine Model Of Preterm Infection/Inflammation-Mediated Brain Injury: Effects On MMP, TIMP And TACE Expression  
L Weaver-Mikaere1, HM Gibbons, M Fraser
- Poster 4: Abstract 42  
Repeated Systemic Fetal Administration Of LPS Upregulates The Expression of Tumour Necrosis Factor Alpha (TNF-  $\alpha$  ) In The Periventricular White Matter Of The Preterm Ovine Fetus  
M Fraser, K Henare, PA Mitchell, MD Mitchell
- Poster 5: Abstract 43  
Aldosterone Effects Changes in Lung Compliance in the Ovine Fetal Lung  
Jarret McCartney, Xiaodi Feng, Charles Wood and Maureen Keller-Wood
- Poster 6: Abstract 44  
Melatonin Administration in an Ovine Model of *In Utero* Infection -a Prospect for Neuroprotection?  
Burcu Saglam, Suzanne L. Miller, Graham Jenkin, and Euan M. Wallace
- Poster 7: Abstract 45  
Physiology of the Ovary During Pregnancy, Parturition and Lactation in the Spiny Mouse  
Esther Nitsch, Bree O'Connell, Hayley Dickinson, Alan Tilbrook, David W Walker
- Poster 8: Abstract 46  
Adrenalectomy Causes Increased Progenitor Cell Proliferation in the Late Gestation Fetal Sheep Brain.  
Kim Wamper, Jacob Hollis, Hanna Genee, Nancy Nichols, Jon Hirst, David W Walker

**PROGRAMMED ENHANCED ADIPOGENESIS CONTRIBUTES TO ADULT OBESITY IN GROWHT RESTRICTED OFFSPRING: EVIDENCE FROM EX VIVO ADIPOSE CELL CULTURE**

M. Desai<sup>1</sup>, T.R. Magee<sup>1</sup>, G. Han<sup>1</sup>, M.G. Ross<sup>1</sup>.

<sup>1</sup>*Dept. of Ob/Gyn, LABioMed at Harbor-UCLA Med. Ctr.*

**Introduction:** Insulin, a potent inducer of adipogenesis and lipogenesis, acts via stimulation of the adipogenic transcription factor (peroxisome-proliferator-activated-receptor, PPAR $\gamma$ ) lipogenic transcription factor (sterol regulatory element binding-protein, SREBP1) and lipogenic enzyme (fatty acid synthase, FAS). Obese individuals exhibit upregulation of PPAR $\gamma$ , SREBP1 and FAS, and develop cellular resistance to insulin. In rats, maternal food restriction in pregnancy results in IUGR newborns which develop adult obesity, hyperinsulinemia and insulin resistance. IUGR newborns have low plasma insulin levels, but paradoxically *increased* adipose PPAR $\gamma$  expression. To determine if IUGR adipose tissue has a programmed propensity to adipogenesis and lipogenesis, in the absence of systemic factors, we examined IUGR and Control adipose tissue proliferation, differentiation and signaling in an ex vivo tissue culture.

**Methods:** Control dams received ad libitum food, whereas study dams were 50% food-restricted from pregnancy day 10 to term to produce IUGR newborns. Adipose tissue from 1 day old IUGR and Controls was collected, preadipocytes isolated and grown in absence of insulin. After 48h, preadipocytes were treated with insulin (5, 20 or 40  $\mu$ g/ml) for 24h. Cell proliferation rate (MTT) was determined and cells were extracted and measured for protein expression (Western Blot) of insulin signal molecules. Additionally, preadipocytes were allowed to differentiate and treated with insulin (as above). Adipocyte lipid content (oil-red) and protein expression of PPAR $\gamma$ , SREBP1c and FAS was determined.

**Results:** The basal *preadipocyte* proliferation was significantly increased in IUGR newborns (2-fold). Consistent with this, IUGR preadipocytes exhibited significant upregulation of insulin signaling pathway as evident by increased protein expression of insulin receptor $\beta$  (1.5-fold), insulin substrates (IRS1, 9-fold; IRS2, 8-fold) and AKT (4-fold). In response to insulin, both Controls and IUGR showed dose-dependent increment in proliferation though at all times, the IUGR exhibited enhanced preadipocyte proliferation as compared to Controls. In *differentiated adipocytes*, the basal lipid content was significantly increased in IUGR newborns (1.5-fold). In concert with this, IUGR adipocytes had significantly increased protein expression of PPAR $\gamma$  (5-fold), SREBP1 (1.5-fold) and FAS (6-fold) as compared to Control adipocytes. In response to insulin, IUGR adipocytes continued to exhibit increased lipid content with upregulated PPAR $\gamma$ , SREBP and FAS.

**Conclusions:** The basal upregulation of adipogenic and lipogenic factors in newborn IUGR adipose tissue indicates a programmed obesity phenotype, independent of systemic endocrine influences. Furthermore, IUGR preadipocytes and differentiated adipocytes have enhanced insulin sensitivity which facilitate increased adipocyte proliferation and differentiation with enhanced propensity for lipid storage.

These results indicate that programmed enhanced adipogenesis contributes importantly to the development of adult obesity in IUGR offspring.

**SMALL INCREASES IN FETAL CORTISOL INCREASE EXPRESSION OF ENAC ALPHA IN FETAL LUNG WITHOUT CHANGING EXPRESSION OF OTHER ENAC SUBUNITS OR AQUAPORIN. Xiaodi Feng and Maureen Keller-Wood; Department of Pharmacodynamics, University of Florida, Gainesville, FL USA**

**Introduction:** At parturition, the fetus undergoes significant physiological changes in order to adapt to the ultra-uterine environment. One of the most critical changes is in transition of the fetal lung from secretion to absorption in order to remove fetal liquid and allow exchange of O<sub>2</sub> and CO<sub>2</sub> postnatally. The mechanism of the reabsorption of lung liquid involves reabsorption of sodium across the alveolar epithelium through the activation of epithelial sodium channels (ENaC) and Na,K ATPase $\alpha$ 1. Our objective was to study the effect of cortisol administration on the channels involved in fetal lung fluid absorption, including subunits of ENaC, Na,K ATP $\alpha$ 1 subunit and the aquaporins (AQP) in late gestation fetal sheep. Our previous study of the ontogenetic pattern of these factors showed an increase in ENaC $\alpha$  expression by 120-130days, and we hypothesized that physiologic increases in cortisol would induce expression of ENaC $\alpha$ .

**Methods:** Five pregnant sheep with twin fetus were surgically prepared with arterial and venous catheters on day 120-123 of gestation. In each sheep one twin was treated with a subcutaneous pellet releasing cortisol hemisuccinate to produce cortisol concentrations similar to that expected in the preterm fetus under stress; the other twin was untreated. After six days treatment, the sheep were killed and fetal lungs were obtained. Expressions of mRNA for ENaC $\alpha$ , ENaC $\beta$ , ENaC $\gamma$ , Na,K ATPase $\alpha$ 1 and AQP1 and AQP5 in fetal lungs were measured using real-time PCR. In order to compare expression of the ENaC subunits, standard curves for ENaC  $\alpha$ ,  $\beta$ , and  $\gamma$  were created to allow absolute quantification of expression of each subunit.

**Results:** Expression of ENaC $\beta$  was greater than that of ENaC $\alpha$  or  $\gamma$  (ENaC $\beta$ : 551 $\pm$ 32 copies/ng cDNA, ENaC $\alpha$ : 95 $\pm$ 9 copies/ng cDNA; ENaC  $\gamma$ : 9 $\pm$ 2 copies/ng cDNA in control twins, p<0.001). Cortisol treatment significantly increased cortisol relative to their control fetus (14.3  $\pm$  2.4 nM in cortisol-treated twin as compared to 2.7  $\pm$  0.9 nM in the control twin). There was a significant increase in expression of mRNA of ENaC $\alpha$  in cortisol-treated fetuses as compared to their control twins (152 $\pm$ 23 in cortisol-treated twin compared to 95 $\pm$ 9 copies/ng cDNA in the control twin, p<0.05), but there were no changes in the expression of mRNA for ENaC $\beta$ , ENaC $\gamma$ , AQP1, AQP5 and Na,K ATPase  $\alpha$ 1 between twins.

**Conclusion:** The data suggests cortisol induces ENaC $\alpha$  and therefore plays a central role in the increased sodium channel activity and fluid absorption in late gestation. The ability of a modest increase in cortisol to increase ENaC $\alpha$  suggests that this effect of cortisol may be mediated by activation of MR, and may allow slowing of net lung liquid production as term approaches.

**REPEATED EXPOSURE TO TNF- $\alpha$  IN AN *IN VITRO* OVINE MODEL OF PRETERM INFECTION/INFLAMMATION-MEDIATED BRAIN INJURY: EFFECTS ON MMP, TIMP AND TACE EXPRESSION**

L Weaver-Mikaere<sup>1,3</sup>, HM Gibbons<sup>2</sup>, M Fraser<sup>1,3</sup>

The Liggins Institute<sup>1</sup> and Departments of Pharmacology<sup>2</sup> and Physiology<sup>3</sup>, University of Auckland, Auckland, New Zealand

**Introduction:** Intrauterine infection and inflammation are highly associated with premature delivery, subsequent white matter injury (WMI) and later neurodevelopment impairment in preterm infants; however, the precise pathways involved are poorly understood. There is increasing experimental evidence that inflammatory mediators such as cytokines, in particular tumour necrosis factor alpha (TNF- $\alpha$ ) produced within the brain in response to infection, are strongly associated with cell death in oligodendrocytes, the myelinating cells of the CNS [1]. In addition, these cytokine-mediated events may also be associated with release of matrix metalloproteinases (MMPs). MMPs are vital in many physiological processes. However recently it has been demonstrated that inappropriate activity of these proteinases, particularly the gelatinases (MMP-2 and MMP-9), can have detrimental consequences, as demonstrated by their destructive effects on the extracellular matrix (ECM) in the brain [2]. Given that ECM disruption and increased cytokine levels are frequently observed following injury to the developing brain, we evaluated the association of MMP-2 and -9 with inflammatory-mediated WMI in a preterm ovine *in vitro* model.

**Methods:** Primary mixed glial cultures were derived from preterm (0.65 gestation, term is 147 days) ovine forebrains and subsequently characterised to represent a robust *in vitro* model to study the mechanisms of infection/inflammation-mediated WMI. Infection/inflammation was induced in cultures by exposure to a chronic treatment regime of 100ng/mL of ovine recombinant TNF- $\alpha$  for 5 days. The time-course and duration of the *in vitro* effects of TNF- $\alpha$  treatment on genes encoding the gelatinases, MMP-2 and -9, their endogenous inhibitors, tissue inhibitor of metalloproteinases (TIMP-1 and -2) as well as TNF- $\alpha$  converting enzyme (TACE) were determined using quantitative real-time PCR. Gelatinase activity of MMP-2 and -9 was assessed by gelatin zymography.

**Results:** Gene expression was markedly increased and paralleled observations of increased cell death in treated cultures by 72 hours for MMP-2 ( $P < 0.001$ ) and 12 hours for MMP-9 ( $P < 0.001$ ). Activity of MMP-2 progressively increased across time while MMP-9 activity first increased and later decreased to levels of controls. TIMP-1 expression following TNF- $\alpha$  exposure increased after 96 hours ( $P < 0.01$ ). The increase in TIMP-2 expression did not reach significance across the 5 days and expression of TACE was persistently increased in all treated cultures ( $P < 0.001$ ).

**Conclusions:** In conclusion, mixed glial cultures established from the fetal ovine forebrain provide a highly reproducible model of preterm WMI. Our data of TNF- $\alpha$  induced changes in expression of MMPs *in vitro* suggest that MMP-2 and MMP-9 likely contributes to injury in response to infection/inflammation.

**References:**

1. Fraser, M et al. Repeated systemic fetal administration of LPS upregulates the expression of tumour necrosis factor alpha (TNF- $\alpha$ ) in the periventricular white matter of the preterm ovine fetus. Fetal and Neonatal Physiological Society. 2009. Lake Arrowhead, USA.
2. Ranasinghe, HS et al. Proteolytic activity during cortical development is distinct from that involved in hypoxic ischemic injury. Neuroscience, 2009. **158**(2): p. 732.

**REPEATED SYSTEMIC FETAL ADMINISTRATION OF LPS UPREGULATES THE EXPRESSION OF TUMOUR NECROSIS FACTOR ALPHA (TNF- $\alpha$ ) IN THE PERIVENTRICULAR WHITE MATTER OF THE PRETERM OVINE FETUS**

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**Introduction:** Intrauterine infection is now recognised as a major antecedent of white matter injury (WMI) in the preterm infant brain, which can manifest later as cerebral palsy or as varying degrees of cognitive dysfunction. WMI in these infants is characterised by damage to immature oligodendrocytes, and has been linked both to microglial activation and to elevated levels of TNF- $\alpha$ , IL-6 and IL-1 $\beta$ . We have developed a fetal sheep model of diffuse and focal WMI, based on repeated administration of *E. coli* lipopolysaccharide (LPS) as the stimulus for an inflammatory response.

**Methods:** Surgery to implant fetal vascular catheters was performed on pregnant ewes carrying single fetuses at d89-90 of gestation. Fetuses received repeated IV injections of LPS (100ng/kg estimated fetal weight/day) between d95 and d99. Plasma levels of the pro-inflammatory cytokine TNF- $\alpha$  were determined in fetal arterial blood samples taken between d94 and d104. At d105 ewes and fetuses were euthanised and fetal brain tissue samples collected for histological analysis.

**Results:** Five days after final administration of LPS to fetuses we observed a pattern of WMI similar to that seen clinically, ranging from focal to diffuse injury within the periventricular region, impairment of white matter (CNPase; marker for immature/mature oligodendrocytes) tracts, and thinning of the corpus callosum, characterised by oligodendrocyte disruption and microglial proliferation in the surrounding and sub-cortical white matter. Systemic LPS administration was associated with increased TNF- $\alpha$  immunoreactivity in glial cells of the periventricular white matter. Although a progressive rise in fetal circulating plasma TNF- $\alpha$  concentrations was observed between days 97 and 103 (day two of treatment to third day following final dose of LPS) concentrations were not significantly higher following LPS exposure at any time point.

**Conclusions:** In conclusion, this study shows that non-lethal exposure to a repeated dose of LPS can adversely affect developing white matter ranging from focal to diffuse injury and induce a cytokine-mediated immune response involving TNF- $\alpha$  within the periventricular white matter, providing further support for the hypothesis that proinflammatory cytokines may play a role in the genesis of infection-related white matter injury.

**ALDOSTERONE EFFECTS CHANGES IN LUNG COMPLIANCE IN THE OVINE FETAL LUNG**

Jarret McCartney, Xiaodi Feng, Charles Wood and Maureen Keller-Wood; Departments of Pharmacodynamics and Physiology and Functional Genomics. University of Florida, Gainesville, FL USA

In mammalian pregnancy, the in utero cortisol surge that occurs shortly before parturition stimulates lung maturation through glucocorticoid receptor (GR) –dependent effects. Antenatal administration of synthetic glucocorticoids significantly decreases respiratory distress in preterm fetuses through induction of surfactant proteins. We have found that the mineralocorticoid receptor (MR) are also abundantly expressed in fetal lungs, as are several genes known to be induced by MR action in the adult kidney, including epithelial sodium channel subunit alpha (ENaC $\alpha$ ) and Na,K ATPase $\alpha$ 1. However, the role of MR activation in fetal lung maturation is not clear. The purpose of this study was to determine if administration of a MR agonist would increase lung maturation and compliance in ovine fetuses at 130d gestation.

Fetuses (20 twin, 6 singleton pregnancies) were catheterized at 122-124d gestational age. At 127-131d gestation, fetuses were infused for 48 hour with either the MR specific agonist, aldosterone (0.2 mg/48h; n=6), the GR specific agonist, betamethasone (0.24/48h; n=5), or aldosterone and betamethasone combined (n=5). Additional groups received an increased betamethasone dose, a .25mg initial bolus followed by infusion of betamethasone (total dose 0.75 mg/48h; n=4), or this dose of betamethasone with aldosterone dose (n=5). In twin pregnancies one fetus received corticosteroids; untreated fetuses served as controls (n=14 after exclusion of any hypoxic twins). These doses were chosen to simulate the physiological activation of MR/GR by endogenous cortisol during fetal stress, were based on the clearance rates and affinity of these corticosteroids for MR and GR respectively. After 48 hours of infusion, fetal blood gas values and blood pressures were measured and the ewes and fetuses were euthanized. In situ lung compliance was then determined by measurement of the change in tracheal pressure produced by inflation of the lungs by 4-5 10ml steps of air injected into the trachea. In some of the fetuses, the lungs were allowed to equilibrate to baseline pressure and the pressure measurements were repeated (n=4-5/group).

Although there were significant overall effects of volume to increase pressure, there was no effect of volume on pressure measured during the first series of inflations in the aldosterone-treated fetuses. Aldosterone alone resulted in a leftward shift in the volume-pressure graph, as there was a lower pressure at each inflation volume. At 10, 30, and 40 ml, the pressure produced in the aldosterone-treated fetuses was significantly less than in the control fetuses. Somewhat surprisingly, there was no significant effect of betamethasone at either dose, although betamethasone at the low dose significantly attenuated the response to aldosterone alone. However, the combination of aldosterone and the higher doses of betamethasone is similar to that of aldosterone alone. The results suggest an effect of aldosterone on lung compliance in the preterm ovine lung. This effect occurs at a smaller mineralocorticoid dose than glucocorticoid dose, suggesting that the mechanism involves induction of MR-specific factors. Further studies to identify molecular targets of MR and possible effects on lung structure and/or genes involved in lung fluid resorption are underway.

## MELATONIN ADMINISTRATION IN AN OVINE MODEL OF *IN UTERO* INFECTION - A PROSPECT FOR NEUROPROTECTION?

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<sup>1</sup>Department of Obstetrics & Gynaecology and <sup>2</sup>Monash Immunology & Stem Cell Laboratories, Monash University, Clayton, Victoria.

**Introduction:** Hypoxia and hypotension are commonly associated with infection in human neonates and observed in fetal animal models of LPS-induced in utero infection. Systemic hypoxia induces the cellular production of reactive oxygen species (ROS) which have highly detrimental effects on the developing brain - initiating hypomyelination, blood-brain barrier (BBB) breakdown, lipid peroxidation and cell death. The pineal hormone melatonin (MLT) is an antioxidant and powerful scavenger of ROS. We hypothesized that maternally administered MLT could reduce hypomyelination, lipid peroxidation and cell death in the fetal brain in response repeated low-dose endotoxin.

**Methods:** Surgery was performed on singleton-bearing ewes with fetal gestational age 104-110 days (term ~147 days). Catheters were inserted into the fetal femoral artery and vein, and a flow probe placed around the carotid artery. After surgery, ewes were randomly assigned to receive either a continuous infusion of 1) MLT (1 mg/hour; n=5) beginning 6 hours prior to the administration of the 1<sup>st</sup> LPS; 3) or vehicle (saline; n=5) into the maternal jugular vein. In all experiments, irrespective of maternal treatment, the fetus received three daily i.v. infusions of LPS (100ng/kg). Fetal blood-gases and pH were monitored daily; mean arterial blood pressure (MAP), heart rate (HR) and carotid blood flow (CaBF) were recorded continuously. Fetal plasma samples were taken to assess inflammatory and oxidative stress responses by measuring fetal arterial concentrations of TNF- $\alpha$ , IL-6 and malondialdehyde (MDA). Fetal brains were collected at post mortem (72 hours after the administration of LPS #1), for immunohistochemical evaluation of apoptotic cells, lipid peroxidation, blood-brain barrier permeability and periventricular white matter (PVWM) injury using TUNEL, 4-HNE, albumin and CNPase antibodies, respectively.

**Results:** In response to the repeated low-dose LPS, fetuses experienced transient acidaemia, hypoxia and hypercapnia, as well as significantly reduced MAP ( $p = 0.03$ ), significantly elevated HR ( $p = 0.02$ ) and significantly elevated CaBF ( $p = 0.03$ ). Furthermore, these fetuses had increased circulating levels of TNF- $\alpha$ , IL-6 and MDA in response to the administration of repeated low-dose endotoxin. Maternal MLT administration increased fetal melatonin levels approximately 100-fold, but did not affect the LPS-induced changes in fetal blood-gas, hemodynamic responses, fetal TNF- $\alpha$  and IL-6 levels. However, maternal MLT administration did significantly mitigate circulating concentrations of MDA in response to repeated low-dose endotoxin ( $p = 0.02$ ). Maternal MLT administration significantly reduced the 4-HNE-immunoreactivity in the PVWM, but not the TUNEL-, 4-HNE-, albumin- and CNPase-immunoreactivity.

**Discussion/Conclusion:** While the administration of MLT to the pregnant ewe significantly reduced fetal systemic and brain levels of lipid peroxidation (MDA and 4-HNE, respectively), which occurs after exposure to repeated low-dose endotoxin administration, it did not reduce the fetal inflammatory response, nor improve hypomyelination, cell death and BBB breakdown in the PVWM. Taken together, these results demonstrate that MLT is an effective antioxidant, but is not effective in reducing the brain injury that results from fetal endotoxin exposure.

**Physiology of the ovary during pregnancy, parturition and lactation in the spiny mouse**Esther Nitsch<sup>1</sup>, Bree O'Connell, Hayley Dickinson, Alan Tilbrook, **David W Walker**<sup>1</sup>University of Maastricht, Netherlands; Department of Physiology, Monash University, Clayton, Australia, 3800

**Introduction:** The spiny mouse is a rodent species with a relatively long gestation that exhibits a postpartum estrus immediately (within 24h) after parturition. The mechanism of delivery (labor) is not known in the spiny mouse, nor is it known whether the placenta (as in the human) or the ovary (as in the mouse) maintains pregnancy. We sought to answer these questions in the current study in the spiny mouse.

**Methods:** Pregnant spiny mice underwent ovariectomy or sham surgery at 30 days gestation (n=6). Animals were monitored and their weight recorded every 12 h. To describe the structural changes in the pregnant ovary dams were sacrificed throughout pregnancy, a blood sample taken and the left and right ovary collected. Ovaries were processed to paraffin wax, sectioned, stained with H&E and photomicrographs taken. Radioimmunoassays will be used to measure the local and circulating reproductive hormones, progesterone, estrogen, FSH and LH throughout pregnancy.

**Results:** All dams that underwent the ovariectomy procedure failed to maintain pregnancy and aborted within 24-48 h post-operatively. Consistent with the ovary being essential for the maintenance of pregnancy, large corpus lutea/albicans were present in the ovary throughout pregnancy. At all stages of pregnancy, follicles at all stages of maturation were present in the ovary.

**Discussion/Conclusion:** Pregnancy appears to be maintained in the spiny mouse by ovarian function, presumably by active corpus lutea. At the same time, the ovary supports continuing maturation of follicles in preparation for resuming ovulation, which can occur within 24 h of delivery. This postpartum estrus occurs even though the dam is lactating, supporting 2-5 offspring. Our ongoing studies, including hormone analysis, are directed at understanding how this is possible in what would traditionally be considered a conflicting hormonal environment.

## Adrenalectomy causes increased progenitor cell proliferation in the late gestation fetal sheep brain.

**Kim Wamper<sup>1</sup>, Jacob Hollis, Hanna Genee, Nancy Nichols, Jon Hirst<sup>2</sup>,  
David W Walker**

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<sup>2</sup>Department of Pharmacology, University of Newcastle, Australia.

**Introduction:** The number of neurons and glia in the mature nervous system is not only a function of cell proliferation, but also of programmed cell death (1). The molecular mechanisms regulating the entry and exit of cells into the cell cycle is determined by a balance of intrinsic and extrinsic pathways. Glucocorticoids are essential for the maturation and normal development of several fetal organs, including the brain, and numerous studies report that high levels of glucocorticoids decrease progenitor cell proliferation in the CNS (2,3). To understand the role of glucocorticoids in brain development, the present study assessed the effect of adrenalectomy (ADX) on the proliferation and migration of progenitor cells in the proliferative zones of the late gestation fetal sheep brain.

**Methods:** Pregnant ewes (n=10) underwent surgery at ~118 days gestational age (GA) for insertion of fetal venous and arterial catheters and, depending on treatment group, for fetal ADX (n=5). At 134 and 137 days GA BrdU (750mg in 30ml 8 mM NaOH) was infused at 20 ml/h into the fetal venous catheter. Fetal arterial blood samples were taken before, during and after the BrdU infusion for blood gas/pH analysis and to determine plasma cortisol concentrations by radioimmunoassay. Fetal brains were obtained at 140 days GA to determine *previous* (BrdU-labeled cells) and *concurrent* (Ki67-labeled cells) cell proliferation using immunohistochemical staining. The cerebral subventricular zone (SVZ), hippocampal dentate gyrus (DG), and cerebellar external granular layer (EGL) and adjacent regions were examined in detail. The density of single and double-labeled cells was determined using ImageJ and AxioVision LE Rel.4.5 software. The Mann-Whitney U-test was used for statistical analysis.

**Results:** Plasma cortisol concentrations were significantly lower in ADX fetuses after surgery and immediately before autopsy; BrdU infusion had no effect on plasma cortisol. After ADX there was a significant increase in the density of BrdU-positive cells *within* the SVZ (p=0.01), DG (p<0.001) and EGL (p=0.003) and in regions immediately *outside* each of these proliferative zones (p<0.001, all regions). In contrast, the density of Ki67-positive cells, actually undergoing division, was significantly decreased *within* the proliferative zones of SVZ (p=0.01) and EGL (p<0.001), whereas they were increased in DG (p<0.05) and in regions immediately *outside* each proliferative zone.

**Discussion:** This data suggests that normal levels of cortisol not only suppress cell proliferation, but also limit the number of cells leaving the proliferative zones in the late gestation fetal brain. Thus, after ADX there were fewer mitotic cells within SVZ and EGL, and a greater number in the DG and in regions immediately adjacent to these zones.

### References

1. Dehay C et al. (2007) Nature Rev Neurosci 8: 438-50..
2. Crochemore C et al. (2002) FASEB J 16: 761-70.
3. Quiros I et al. (2008) J Steroid Biochem Molec Biol., 110: 116-24.

## SESSION VII: (Clinical/Translational)

Chair: David Walker

Wednesday, September 30<sup>th</sup>

- 8:00am – 8:15am      Abstract 47:  
Development of Venous Liver Perfusion in Macrosomic Non-Diabetic Fetuses  
Joerg Kessler, Svein Rasmussen, and Torvid Kiserud
- 8:15am – 8:30am      Abstract 48:  
Cerebral Oxygenation and Epileptiform Activity after the Norwood Procedure  
for Hypoplastic Left Heart Syndrome  
Paul P Drury, Laura Bennet, John S Beca, Laura-Claire Whelan,  
Mark Gunning, and Alistair J Gunn
- 8:30am – 8:45am      Abstract 49:  
Magnetic Resonance T2\* Measurements of Oxygen Saturation in Blood Samples  
of Adult and Fetal Sheep, and of Adult Humans  
Hobe J. Schröder, Arlin Blood, Gordon G. Power, Brenda Bartnik-Olson, and  
Barbara Holshouser
- 8:45am – 9:00am      Abstract 50:  
Decision-Support Tool in Fetal Heart Rate Monitoring  
J T Parer and Emily F Hamilton
- 9:00am – 9:45am      Abstract 51:  
Human Amnion Epithelial Cells: Potential Clinical Application  
S Murphy, S Rosli, R Acharya, R Lim, G Jenkin, and E Wallace
- 9:45am – 10:00am      Abstract 52:  
Isolation and Characterization of Ovine Amniotic Epithelial Cells for use in Pre-  
Clinical Trials  
Jitong Guo, Tony Goldschlager and Graham Jenkin

**DEVELOPMENT OF VENOUS LIVER PERFUSION IN MACROSOMIC NON-DIABETIC FETUSES**

Joerg Kessler, Svein Rasmussen, Torvid Kiserud  
University of Bergen, Department of Clinical Medicine, Bergen, Norway

**Introduction:** Mechanisms regulating fetal growth are incompletely understood. Insulin-like growth factors (IGF) synthesized in the fetal liver stimulate proliferation in fetal organs. Experimental studies have shown that this IGF-synthesis is linked to the magnitude of umbilical venous perfusion of the fetal liver. Here we study the development of the fetal venous liver perfusion during the second half of non-diabetic pregnancies developing macrosomic at birth.

**Materials and Methods:** 39 healthy women, who previously had delivered children with birthweights > 4200 g, were recruited to a longitudinal study after informed consent. Ultrasound examinations were done at monthly intervals during the second half of pregnancy. Blood flow velocities and vessel diameter were measured at the intra-abdominal portion of the umbilical vein, the inlet of the ductus venosus, the left portal vein and the main portal stem. Volume blood flow was calculated and normalized for fetal weight using birthweight centiles. Mean curves for the study population were established based on regression analysis and compared with a reference population.

**Results and discussion:** 25/39 neonates had a birthweight above the 80<sup>th</sup> centile and were included in the analysis. Median birthweight was 4580 g (range 4200-5300) at median gestational age 41.14 weeks (range 38.00-42.29). These macrosomic fetuses showed increased blood flow (also when normalized for weight) in the umbilical, left portal and main portal veins. However, blood flow through the ductus venosus and its shunt fraction was not different from the reference population. The distribution between the left and right liver lobes was maintained equal to that in the reference population. However, a difference was seen in the contribution of portal (splanchnic) compared to umbilical blood to the fetal liver. In contrast to the umbilical contribution, the portal contribution to total venous liver flow was lower than that in the reference population. The present longitudinal study also demonstrated that the blunted growth of venous flows conventionally seen in the reference group was substituted by a continued increase of venous flows throughout the third trimester in the macrosomic fetuses.

**Conclusions:** In contrast to the reference population, the fetuses that develop high birthweight demonstrate a continuous growth of venous liver perfusion, predominantly of umbilical origin, until term. The results support the concept that venous liver perfusion is a determinant for fetal growth. This mechanism seems particularly prominent during the third trimester of pregnancy.

**CEREBRAL OXYGENATION AND EPILEPTIFORM ACTIVITY AFTER THE NORWOOD PROCEDURE FOR HYPOPLASTIC LEFT HEART SYNDROME**

Paul P Drury<sup>1</sup>, Laura Bennet<sup>1</sup>, John S Beca<sup>2</sup>, Laura-Claire Whelan<sup>2</sup>, Mark Gunning<sup>1</sup>, Alistair J Gunn<sup>1,3</sup>

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**Introduction:** Hypoplastic left heart syndrome affects around 2/10000 live births and is one of the most severe cyanotic congenital heart defects (CHD), accounting for 7.5% of newborn CHD and 25% of the deaths from CHD in the first week of life. Three stage palliation, beginning with the Norwood procedure, has significantly improved survival rates but has not reduced the risk of neurodevelopmental handicap. Currently, there are no means of identifying this injury when it may still be treatable. Near-infrared spectroscopy (NIRS) has shown promise for identifying compromised cerebral oxygenation, but requires further validation. The present study related post-operative cerebral oxygenation and oxygen extraction to the development of delayed electrographic seizures in term infants undergoing the Norwood procedure.

**Materials and Method:** Near-infrared spectroscopy (NIRS) parameters, arterial oxygen saturation (SaO<sub>2</sub>), EEG and mean arterial blood pressure (MAP) were recorded from 4 to 18 h after surgery. Intracerebral oxygenation was determined as the tissue oxygenation index (TOI) and fractional tissue oxygen extraction (FTOE = [SaO<sub>2</sub> - TOI] / SaO<sub>2</sub>). This group was then compared with nineteen term infants with Transposition of the Great Arteries (TGA, a homogenous defect).

**Results:** Eleven term infants (weight  $3.4 \pm 0.5$  kg, mean  $\pm$  SD) were studied at  $5.8 \pm 1.5$  days of age. TOI was  $45.3 \pm 3.8\%$  at 4h and progressively increased to  $55.3 \pm 2.8\%$  by 18h after bypass. FTOE was  $0.44 \pm 0.04$  at 4h and progressively fell to  $0.34 \pm 0.02$  at 18h (mean  $\pm$  SEM). Preliminary evidence suggests all infants had epileptiform activity after surgery, and most had electrographic seizures. Compared to infants with TGA (n=19) TOI was significantly lower ( $p = 0.013$ , repeated measures ANOVA), FTOE was not significantly different ( $p = 0.363$ ), and more electrographic seizures were seen after the Norwood procedure than correction of TGA.

**Conclusion:** Poor cerebral oxygenation and electrographic seizures are common after the Norwood procedure, however, FTOE was similar, consistent with preservation of oxygen metabolism by greater extraction. Speculatively, increased electrographic seizures may reflect unilateral perfusion during the Norwood procedure leading to greater focal injury, not detected by changes in global metabolism.

## MAGNETIC RESONANCE T2\* MEASUREMENTS OF OXYGEN SATURATION IN BLOOD SAMPLES OF ADULT AND FETAL SHEEP, AND OF ADULT HUMANS

Hobe J. Schröder<sup>1</sup>, Arlin Blood<sup>1</sup>, Gordon G. Power<sup>1</sup>, Brenda Bartnik-Olson<sup>2</sup>, Barbara Holshouser<sup>2</sup>  
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**Introduction:** At present, blood oxygen saturation (sO<sub>2</sub>) of the human fetus *in utero* can be measured only invasively. The transverse relaxation time T2\* of blood depends<sup>1-5</sup> on the de-oxyhemoglobin content of red blood cells (RBC). This study aims to establish the precision of sO<sub>2</sub> measurement using non-invasive MRI in sheep and human blood samples for future use in *in-vivo* studies (e.g. umbilical vein of chronic fetal sheep).

**Materials and Methods:** Heparinized blood from sheep (16 adult, 6 fetuses) and from adult human volunteers (3 female, 3 male) was oxygenated at four different levels (sO<sub>2</sub> 10 to 100 %; Radiometer OSM3), and hematocrit (Hct) was about 43% (human) or varied from 26 to 51 % (sheep). Usually four samples for each subject (3 ml syringes, 9 mm ID) were imaged at 3T using a 12-channel head coil (Tim/Trio, Siemens Medical Solutions). To calculate T2\*, a multiecho, spoiled-gradient recalled echo acquisition was used with echo delay times TE from 2.3 to 40 msec, TR=400 msec, slice 10 mm thick, and a 0.5 x 0.5 mm in-plane resolution. Signal intensity SI was measured in a circular ROI (0.3 cm<sup>2</sup>) in cross sections. T2\* relaxation rates were calculated using  $R2^* = 1/T2^*$  (sec<sup>-1</sup>) which was derived from the slope of log (SI) versus TE with r<sup>2</sup> as a measure of linearity (r<sup>2</sup>). The square root of R2\* (SQRT(R2\*)) was linearly related to oxygen saturation. **Results:** In humans,  $SQRT(R2^*) = 20.3 - 0.160 sO_2$  (r<sup>2</sup>=0.97), in adult sheep  $SQRT(R2^*) = 9.9 - 0.071 sO_2$  (r<sup>2</sup>=0.86) with data selected (r<sup>2</sup>>0.99, n=16) and adjusted for Hct. The adjustment removed the bivariate correlation (sO<sub>2</sub>, Hct) with SQRT(R2\*) of the sheep raw data. Bland-Altman-plots showed a level of precision at ±10% sO<sub>2</sub> for human and at ±20% sO<sub>2</sub> for adult sheep. Adult and fetal sheep blood were not different. **Discussion:** The distinct difference of regression coefficients between sheep and human blood by a factor of 2.3 was unexpected. It is likely caused by the differences in RBC haemoglobin content (MCH) (human adult: 29 pg, sheep adult: 13 pg)<sup>6</sup> and hematocrit<sup>1</sup> (human: 42%, sheep: 35%)<sup>6</sup>. Sheep term fetal MCH (12.8 pg) and Hct (48%)<sup>6</sup> are close to adult values which explains the similarity of maternal and fetal blood in sheep. For term human fetal blood (37.5 pg, Hct 51%)<sup>6</sup>, the regression coefficient is predicted to be increased above that of adult humans from 0.16 to 0.21 but experimental data are not available.

**Conclusions:** MR T2\* measurements may prove useful to measure the oxygen saturation in large blood vessels of the human fetus *in-utero*.

**References:** 1. Thulborn KR et al, Biochim Biophys Acta, 714, 265 (1982) 2. Wright GA et al, JMRI 1, 275 (1991) 3. Spees WM et al, Magn Res Med, 45, 533 (2001) 4. Lee T et al, Proc Intl Soc Mag Reson Med 11 (2003) 5. Zhao JM et al, Magn Res Med 58, 592 (2007) 6. Dittmer DS (ed) Blood and other body fluids, Biological Handbooks, Fed Am Soc Exper Biol, Washington (1961)

## STANDARDIZED INTERPRETATION AND MANAGEMENT WITH A COMPUTERIZED DECISION-SUPPORT TOOL IN FETAL HEART RATE MONITORING

J T Parer<sup>(1)</sup> and Emily F Hamilton<sup>(2)</sup>

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**Introduction:** The utility of FHR monitoring in clinical practice continues to be impeded by lack of consensus in interpretation of FHR patterns, their association with the risk of acidemia, and guidelines for management. This persists despite the acceptance of NICHD standardized nomenclature, and despite the presence of much evidence for the association of acidemia with reduced or absent FHR variability, the relationship of depth and duration of decelerations to acidemia, the natural evolution of patterns with ameliorating techniques, and the integration of this evidence into a system of management which is tentatively clinically acceptable, and testable for utility.

**Materials & Methods:** We have developed a 5-tier colour coded system in which all 134 possible FHR combinations of characteristics are classified, and which incorporates the risk of fetal acidemia and risk of progression of the pattern to a more serious one (Parer & Ikeda, Am J Obstet Gynec 2007;197:26.e1-6). This algorithm also includes practical guidelines for clinical management, which can be modified for application to a hospital's specific facilities and logistics.

**Results:** We have tested the feasibility of the system by showing that experienced obstetrical providers agree amongst themselves with the classification to within 1 colour code in >80% of cases. We have also demonstrated its utility by showing that the incidence of fetal acidemia <7.15 decreased from 1.5% to 0.2%, and base excess from 1.8% to 0.3% in the 2 years following its introduction into a private clinic in Osaka (Ikeda, Parer, Noda et al, SMFM, 2009). In the feasibility study above, we used a commercially available, FDA cleared device, CALM Patterns which includes computer analysis of baseline FHR, variability, accelerations, and decelerations (type, depth and duration) for real time analysis of FHR patterns. The quantitated findings were then combined according to the definitions in the colour-coded 5-tier Framework system, where automated alerts could act as decision support tools for application of the hospital-specific guidelines for management interventions. Assessments by the computer based system were very similar to the clinicians as measured by proportions of agreements and weighted kappa scores.

**Conclusions:** These studies suggest that that this rule-based approach can be effectively used by clinicians for interpretation, and that >80% agreement within 1 colour code is clinically acceptable. The performance of the computer midway between the experts suggests it may be of value as a decision-support device in conventional clinical practice.

**HUMAN AMNION EPITHELIAL CELLS: POTENTIAL CLINICAL APPLICATION**S. Murphy<sup>1,2</sup>, S. Rosli<sup>1</sup>, R. Acharya<sup>1</sup>, R. Lim<sup>1</sup>, G. Jenkin<sup>2</sup>, E. Wallace<sup>1</sup><sup>1</sup>Department of Obstetrics and Gynaecology, <sup>2</sup>Monash Immunology and Stem Cell Laboratories, Monash University, Australia[Sean.Murphy@med.monash.edu.au](mailto:Sean.Murphy@med.monash.edu.au)

**Introduction:** Human amnion epithelial cells (hAECs) are easily accessible and do not have the limitations of adult and embryonic stem cells for their potential clinical application. They are a heterogeneous population positive for stem cell markers and display multi-lineage differentiation potential, forming cells of the endoderm (liver, lung epithelium), mesoderm (bone, fat), and ectoderm (neural cells)<sup>1</sup>. They have a low immunogenic profile and possess potent immunosuppressive properties<sup>2</sup>. Collectively, these findings indicate that hAECs may elicit minimal immune recognition following transplantation to an allogeneic recipient. When administered to mice, following bleomycin induced lung injury, hAECs reduce fibrotic damage, reduce gene expression of pro inflammatory cytokines, increase gene expression of anti inflammatory cytokines and appear to engraft and differentiate into alveolar type 1 and 2 cells<sup>3</sup>. In an in utero ventilation model in fetal sheep, our preliminary results indicate that administration of hAECs also reduces inflammation and fibrosis, thus providing a potential cellular therapy in the treatment of prematurely born infants. We have also evaluated the capacity of xenogeneic hAECs to safely and efficaciously promote osteogenesis in the cervical interbody space in a sheep model of spinal fusion. Hence hAECs may be a valuable source of cells for cell therapy.

**Aim:** To demonstrate feasibility of animal product-free methods of isolation and culture according to current guidelines on cell preparation for clinical use.

**Methods:** An animal product-free cell isolation method was developed and compared to traditional animal product-containing methods. Total cell yield and viability was determined by cell counts and trypan blue exclusion. Purity was established through FACS analysis of epithelial (EpCAM) and mesenchymal (CD90, CD105) marker expression. Animal product-free cryopreservation and growth media were developed and compared to conventional serum-based media. Post-thaw viability, recovery of cell metabolism, and proliferation rates were determined. hAECs were analysed, after 5 passages, by karyotype analysis, cell cycle distribution and changes to telomere length. hAEC were differentiated into lineages of the three primary germ layers and specific markers analysed using PCR, immunocytochemistry and histology.

**Results:** The method developed was comparable to established animal product-containing methods, producing an average yield of  $120 \pm 40 \times 10^6$  hAEC per amnion with average viability of  $83 \pm 4\%$ . Isolated populations were 92% EpCAM positive with  $<1\%$  mesenchymal cell contamination. After 5 passages, hAEC displayed normal karyotype, cell cycle distribution and long telomeres, suggesting that hAEC are unlikely to be tumorigenic. Multipotent differentiation potential of hAEC was demonstrated by induction of neural (MAP2, GFAP, Nestin), bone (osteocalcin, osteonectin), fat (PPAR $\gamma$  LPL), and lung epithelial (SP-C, CC10, Nkx2.1) gene expression as well as by immunocytochemical and histological methods.

**Discussion and Summary:** We have now optimised an animal product-free method for efficient isolation and cryopreservation of hAECs suitable for clinical therapies. Term amnion is an attractive alternative source, to cells derived from the embryo or adult, for the derivation of pluripotent cells for research and possible clinical application.

**References:**

1. Ilancheran *et al.*, Biol Reprod 77: 577-588 (2007).
  2. Li *et al.*, Invest Ophthalmol Vis Sci 46 : 900-907 (2005).
  3. Moodley *et al.*, Mater Medical Research Institute Annual Stem Cell Symposium (2009)
- This work was supported by NH&MRC Project Grant No. 491145.

## ISOLATION AND CHARACTERIZATION OF OVINE AMNIOTIC EPITHELIAL CELLS FOR USE IN PRE-CLINICAL TRIALS

Jitong Guo, Tony Goldschlager and Graham Jenkin

Monash Immunology and Stem Cell Laboratories, Monash University, Clayton, Victoria, Australia

**Introduction:** The amnion is the inner of two membranes encasing the amniotic fluid in which the fetus is suspended during gestation. The placenta and associated membranes are now recognized as important sources of pluripotent cells. Our, and others, previous studies have shown that human amniotic epithelial cells (hAECs), derived from term delivered gestational tissue, display key features of pluripotent stem cells, are capable of self renewal, retain considerable plasticity, do not form teratomas, have restricted expression of major histocompatibility (MHC) antigens and appear to suppress lymphocyte proliferation (Ilancheran et al., 2007; Li et al., 2005). Collectively, these findings indicate that hAECs may elicit minimal immune recognition following transplantation to an allogeneic recipient in their native form, or even after differentiation down a specific lineage.

There have been no reports in the literature of isolation of epithelial cells from ovine amnion. If ovine amnion epithelial cells (oAECs) have similar properties to those that we have demonstrated in the human, it may be possible to exploit their plasticity, trophic, anti-inflammatory, non-tumorigenic and immune-privileged features to develop novel stem cell based therapies.

**Materials and Methods:** Ovine amnion was collected from sheep undergoing caesarian section near term. The tissue was digested twice with TrypLE for 30 min. Dispersed cells were washed and cultured in DMEM/F12 containing 15% FBS and supplements. The resulting ovine amnion-epithelial cells (oAECs) were fixed and then interrogated by immunocytochemistry for pluripotent stem cell markers, Oct-4, SSEA-1, SSEA-3, SSEA-4, Tra-1-60 and Tra-1-80.

**Results and Discussion:** Following isolation and initial culture some epithelial like cells became attached to the petri dish, while others remained suspended in the culture medium. Both groups of cells had the capacity to proliferate and to be passaged up to 6 times. Thereafter, the gross morphology of the cells began to alter, with cells becoming larger but with smaller nuclei, and a slower cell proliferation rate. At passage 1, the oAECs were positive for Oct-4, SSEA-1 and SSEA-4 and weakly positive for Tra-1-60 and Tra-1-80. However, SSEA-3 was undetectable on these cells. We have demonstrated the capacity of these cells to differentiate down the mesodermal lineage. Following culture in differentiation medium for 4 weeks, chondrogenic lineage cells were visualized by staining of Alcian Blue and differentiated cells containing mineral deposits were stained bright red by Alizarin Red Solution, indicating the presence of osteogenic lineage cells.

**Summary:** Term amnion is an attractive alternative source, to cells derived from the embryo or adult, for the derivation of pluripotent cells for research. The *in vivo* capacity for osteogenic and chondrogenic differentiation of oAECs is thus being investigated in pre-clinical trials in sheep.

Ilancheran et al., Biol Reprod 77: 577-588 (2007).

Li et al., Invest Ophthalmol Vis Sci 46 : 900-907 (2005).

This work was supported by the Rural Industries Research and Development Corporation (RIRDC), and NH&MRC Project Grant No. 491145

## SESSION VIII (Endocrinology)

Chair: Dino Guissani

Wednesday, September 30<sup>th</sup>

- 10:30am – 10:45am      Abstract 53:  
Adenosine A1 Receptor Mediated Suppression of Adrenal Activity in Nearterm Fetal Sheep  
EC Jensen, L Bennet, M Fraser, GC Power and Gunn AJ
- 10:45am – 11:00am      Abstract 54:  
Suppression of 5 $\alpha$ -Reductase Isoforms By Betamethasone Treatment in the Placenta and Brain of Normal and Growth Restricted Fetal Guinea Pigs  
Amy A McKendry , Hannah K Palliser, David W Walker, and Jonathan J. Hirst
- 11:00am – 11:15am      Abstract 55:  
Identification and Quantitation of Phase I Hepatic Drug Metabolizing Enzymes in the Fetal, Newborn and Adult Sheep  
Manoja Pretheeban, Caroline Underhill, Eugene Hrycay, Stelvio Bandiera, Geoff Hammond, Wayne Riggs and Dan Rurak
- 11:15am – 11:30am      Abstract 56:  
In the Fetal Rat, the Adrenal and Heart Contain a Circadian Clock  
L Abarzua-Catalan, N Mendez, N Vilches, G Valenzuela, M Seron-Ferre, and C Torres-Farfan.
- 11:30am – 11:45am      Abstract 57:  
Rat Embryonic Hypothalamic Neural Stem Cells (NSC) Response to Trophic Factors: Selective Differentiation Responses to Leptin Insulin  
M Desai, T Li, and MG Ross

**ADENOSINE A<sub>1</sub> RECEPTOR MEDIATED SUPPRESSION OF ADRENAL ACTIVITY IN NEAR-TERM FETAL SHEEP**

Jensen EC<sup>1</sup>, Bennet L<sup>1</sup>, Fraser, M<sup>2</sup>, Power GC<sup>3</sup> and Gunn AJ<sup>1</sup>.

<sup>1</sup>Department of Physiology, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand; <sup>2</sup>The Liggins Institute, the University of Auckland. <sup>3</sup>Center for Perinatal Biology, Loma Linda University School of Medicine, Loma Linda, CA, USA.

**Introduction:** Activation of the hypothalamic-pituitary-adrenal (HPA) axis, with increased fetal adrenocorticotrophic hormone (ACTH) and cortisol concentrations, is a critical response to perinatal hypoxia. There is increasing evidence that these responses are modulated by endocrine and paracrine factors, and in particular that adenosine can both inhibit baseline levels of fetal cortisol and restricts the increase in ACTH and cortisol during acute moderate hypoxia. Since adenosine increases substantially during profound asphyxia, it is possible but untested that counter-intuitively it might restrict the HPA response to severe insults. It is unknown which receptor mediates the effects of adenosine on the HPA axis, however, the adenosine A<sub>1</sub> receptor plays a central role in adaptation to hypoxia. We therefore investigated whether adenosine A<sub>1</sub> receptor activation modulates ACTH and cortisol levels before, during or after 10 min of complete umbilical cord occlusion.

**Material and Methods:** Near-term fetal sheep (118 to 126 days gestation) were randomly allocated to receive saline (n=7) or 8-Cyclopentyl-1,3-dipropylxanthine (DPCPX, an A<sub>1</sub> receptor agonist) (n=7) infused 60 min before and during a 10 min umbilical cord occlusion, followed by 72 h recovery.

**Results:** Fetal ACTH concentrations increased significantly ( $p < 0.01$ ) during occlusion in both groups, and returned to baseline values by 60 min after occlusion. In the saline control group, fetal cortisol and cortisone plasma levels increased significantly ( $p < 0.05$ ) 60 min after the occlusion, and returned to baseline values by 24 h. In contrast, DPCPX was associated with a marked increase in fetal cortisol levels during the pre-occlusion DPCPX infusion, with increased cortisol levels compared with controls sustained up to 72 h.

**Conclusions:** The present study demonstrates that adenosine A<sub>1</sub> receptor activity markedly limits baseline cortisol release but not the ACTH response to asphyxia denoting a direct adrenal effect. A<sub>1</sub> receptor blockade was not associated with any further rise in fetal cortisol levels after asphyxia, albeit levels remained significantly higher than in controls, suggesting that the effect of adenosine is maximal at basal levels, and can be partially overcome by supraphysiological stimuli such as asphyxia. It is intriguing to note that even 10 min of severe asphyxia was not associated with a rise in fetal cortisol during asphyxia although there was a delayed but sustained rise after asphyxia. Presumptively this likely reflects the synthesis time of cortisol, but it implies that the major contribution of the HPA axis is not in immediate fetal adaptation to acute asphyxia, but rather cardiovascular adaptation during recovery.

Suppression of 5 $\alpha$ -reductase isoforms by betamethasone treatment in the placenta and brain of normal and growth restricted fetal guinea pigs

Amy A. McKendry<sup>1</sup>, Hannah K. Palliser<sup>1</sup>, David W. Walker<sup>2</sup>, Jonathan J. Hirst<sup>1</sup>

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Email: jon.hirst@newcastle.edu.au

The placenta has a major role in supplying neuroactive steroids to the fetal brain. The concentrations of these steroids are remarkably high during fetal life and protect the fetal brain from hypoxia/ischemic injury during late gestation (1). 5 $\alpha$ -reductase type 1 and type 2 (5 $\alpha$ R1 and 5 $\alpha$ R2) catalyse a key rate-limiting step in the synthesis of neuroprotective steroids. These isoenzymes are strongly expressed in the placenta, and expression increases with advancing gestation (2), suggesting both 5 $\alpha$ R1 and 5 $\alpha$ R2 contribute to neuroactive steroid synthesis. Betamethasone and growth restriction may suppress neuroactive steroid synthesis in the placenta and/or fetal brain with potential adverse effects on outcome. The objective of this study was to examine if betamethasone treatment influence the expression of 5 $\alpha$ R isoforms in the normal fetus and if this effect was potentiated in growth restricted fetuses.

Placental insufficiency was induced in guinea pigs by ablation of uterine artery branches supplying each placenta at mid-gestation (term 71d). This resulted in intrauterine growth restriction (IUGR) with a marked reduction (35%) in fetal body weight and brain sparing. Control (sham procedure) and IUGR fetuses were treated with vehicle or betamethasone (1mg/kg/day) for 4 days prior to sacrifice (65d). Real time RT-PCR was used to determine 5 $\alpha$ R1 and 5 $\alpha$ R2 mRNA expression in placenta and brain extracts.

5 $\alpha$ R2 expression in the placenta was markedly reduced by betamethasone treatment compared to control (2.5 fold, P<0.002, n=11) whereas 5 $\alpha$ R1 expression was not affected. Expression of 5 $\alpha$ R2 expression was not affected by IUGR alone and was also not reduced when IUGR fetus were treated with betamethasone. 5 $\alpha$ R2 mRNA expression in the brain was reduced betamethasone treatment in male fetuses compared to controls (P<0.01, n=5 and 6), but not in female fetuses. In addition, 5 $\alpha$ R1 in brain expression was increased by IUGR or betamethasone treatment female fetuses (P<0.05, IUGR or betamethasone n=5, control n=6).

The suppression of 5 $\alpha$ R2 expression in the placenta suggests betamethasone may reduce neuroactive steroid output. The betamethasone-induced reduction in 5 $\alpha$ R2 expression in the brain may further reduce neurosteroidogenic capacity and neuroactive steroid levels in the male fetuses, whereas the rise in 5 $\alpha$ R1 expression in the brain of females may maintain concentrations. The suppression in neurosteroidogenic enzyme expression by betamethasone may reduce protective neuroactive steroid levels in the brain and increase the risk of brain injury. This effect may be greater in male fetuses.

(1) Yawno et al 2007 Neuroscience 146: 1726-1733

(2) Vu et al. 2009 Reproduction, Fertility and Development 21:599-607.

## IDENTIFICATION AND QUANTITATION OF PHASE I HEPATIC DRUG METABOLIZING ENZYMES IN THE FETAL, NEWBORN AND ADULT SHEEP

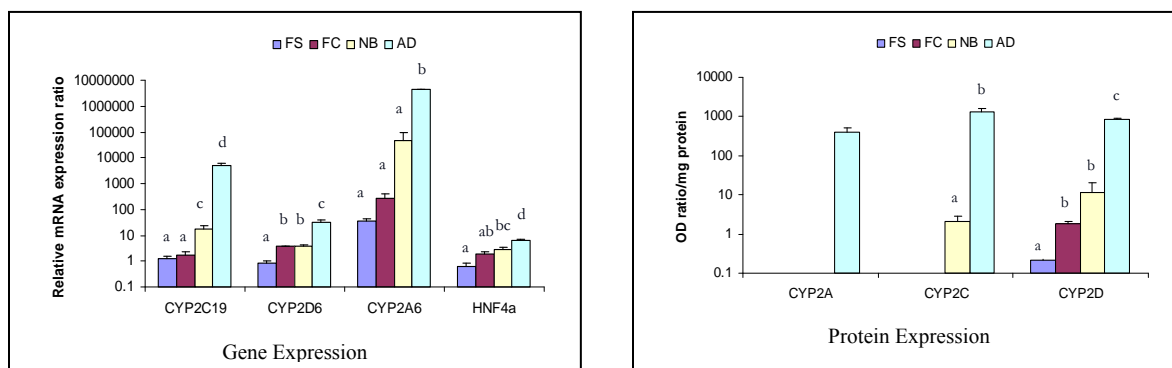
Manoja Pretheeban<sup>1</sup>, Caroline Underhill<sup>1</sup>, Eugene Hrycay<sup>2</sup>, Stelvio Bandiera<sup>2</sup>, Geoff Hammond<sup>1</sup>, Wayne Riggs<sup>2</sup> and Dan Rurak<sup>1</sup>

<sup>1</sup>Department of Obstetrics and Gynecology, Child and Family Research Institute, Faculty of Medicine and <sup>2</sup>Faculty of Pharmaceutical sciences, University of British Columbia, Vancouver, Canada.

**Introduction:** Hepatic drug metabolic enzymes are important for the biotransformation of many drugs that are administered to humans and animals. Cytochrome P450 enzymes (CYP) 2A6, 2C19, 2D6 are enzymes that involved in the metabolism of many drugs. In order to understand whether these drugs are safe enough to be administered during pregnancy and in the postnatal period, knowledge of the expression levels of these enzymes in different developmental stages is important.

**Materials and Methods:** In this study we have used sheep (adult, n=4; newborn, n=3; and fetus, 128 days, n=3) liver tissue to compare the gene and protein expression levels of the above enzymes and a hepatic regulatory factor, HNF4 $\alpha$ , that in adults has been implicated in the activation of several CYP isoforms, as well genes involved in hepatic intermediary metabolism. The effect of antenatal glucocorticoid administration on these enzymes was also studied by infusion of cortisol (0.45mg/h) to another group of fetuses (n=5) for 80 h. Sheep sequences were cloned using human primers and real time PCR was performed to analyze the relative gene expression levels in the above four groups using 18S RNA as house keeping gene. Microsomes were prepared from the liver using differential ultracentrifugation method. Presence of proteins cross reacting with polyclonal human CYP2D6 and CYP2A6 and rat CYP2C11 antibodies was detected by western blot and the relative protein expression levels were measured using human standards.

**Results and Discussion:** Gene and protein expression data are given in the figure below.



Different letters indicate significant difference ( $P < 0.05$ ) between groups

CYP2A6 showed higher variation in gene expression within groups. Western blot revealed two CYP2C like proteins in adults and a single one in newborn sheep. Three bands in adult and two in newborn and fetuses were obtained with human CYP2D6 antibody whereas CYP2A6 antibody gave each a weaker and a stronger band in adults but only a single band in fetus and newborn. Glucocorticoid plays a role in up regulating both the gene and protein expression of CYP2D6 but the effect was limited in magnitude. It may be due to the short duration of cortisol infusion (80h) relative to that of parturition cortisol rise (~20d). Moreover the expression of HNF4  $\alpha$  also follows the expression pattern of CYP2C19 and indicates a possible regulatory role in sheep similar to human. Our study is the first report on the expression of sheep CYP genes 2A6, 2C19, 2D6 and HNF4  $\alpha$ . The findings of this study follow a similar pattern found in the human and indicate that fetal and newborn lambs have a reduced ability to metabolize drugs that are substrates of these CYP isoforms.

**IN THE FETAL RAT, THE ADRENAL AND HEART CONTAIN A CIRCADIAN CLOCK.**

Abarzua-Catalan L<sup>1</sup>, Mendez N<sup>1</sup>, Vilches N<sup>1</sup>, Valenzuela G<sup>2</sup>, Seron-Ferre M<sup>1-3</sup>, Torres-Farfan C<sup>1</sup>.

<sup>1</sup>Programa de Fisiopatología, ICBM, Facultad de Medicina, Universidad de Chile, Santiago. <sup>2</sup>Women's Health, Arrowhead Regional Medical Center, Colton, CA. <sup>3</sup>Universidad de Tarapacá, Arica, Chile.

Organization of the circadian system in the fetus is poorly known. In the adult, the circadian system is organized as a hierarchy of peripheral clocks residing in most organs of the body commanded by a central clock located in the suprachiasmatic nucleus of the hypothalamus (SCN). In altricial species, like the rat, SCN neurogenesis is completed close to birth and oscillatory expression of clock genes and metabolic activity is present at 20 days of gestation (dg) whereas overt circadian rhythms of several physiological variables including corticosterone start postnatally. However recent evidence support the presence of circadian rhythms in some fetal organs during gestation. Indeed, we found that at 18 dg, corticosterone content in the rat fetal adrenal shows a circadian oscillation, with a maximum at 0800-1200h. In addition, the fetal adrenal and the fetal heart present rhythmic expression of clock genes. **Purpose:** To explore whether oscillation of Per2 and Bmal1 in the fetal adrenal gland and fetal heart is sustained in vitro. **Methods:** Pregnant dams (n=24) were euthanized at 20-24 hrs at 18dg by thiopental overdose. Fetal adrenal glands and hearts were dissected, pooled in 30 ml DMEM-F12 and pre-incubated by 6 hrs at 37°C. Then the adrenal and heart pools were aliquoted in culture dishes (8 adrenals/3 heart-per well) in sextuplicate and the explants were collected every 4 hrs for 56 hrs, starting at 0800h. RNA was extracted using a commercial kit and the expression of the clock genes Per2 and Bmal1, Mt1 melatonin receptor in adrenal and heart, StAR (Steroid acute regulatory protein involved in adrenal cholesterol transport, adrenal) and glucocorticoid receptor (GR-heart) were measured by real time-PCR. **Results:** We detected oscillatory expression in culture of the clock genes Bmal1 and Per2 as well as of Mt1 in adrenal (acrophases at 2400 and 1200h respectively) and heart (acrophases at 2000h and 0400h, respectively). Mt1 melatonin receptor was expressed in both tissues along the 56 hours of incubation, with oscillation only in adrenal gland (acrophase at 1600h). On the other hand, StAR presented a circadian rhythm in culture in adrenal gland (acrophase at 1200h), coinciding with that of Per2 and with the timing of maximal plasma corticosterone concentration in the fetus. The fetal heart maintained GR expression along the 56h incubation without demonstrating circadian oscillation.

**Conclusions:** Our data show that, in vitro, the fetal rat adrenal gland and fetal heart sustain Bmal1 and Per2 oscillation, supporting that both fetal tissues are peripheral circadian clocks. We speculate that in vivo the rat fetal adrenal rhythms are synchronized by maternal melatonin, generating a circadian rhythm of glucocorticoids during fetal life. Given that it has been reported that glucocorticoids synchronize peripheral clocks in the adult, we speculate that the fetal heart could be synchronized by fetal glucocorticoids. **Support: Fondecyt-1080649**

**RAT EMBRYONIC HYPOTHALAMIC NEURAL STEM CELLS (NSC) RESPONSE TO TROPHIC FACTORS: SELECTIVE DIFFERENTIATION RESPONSES TO LEPTIN INSULIN**

M. Desai, T. Li, M.G. Ross

*Dept. of Ob/Gyn, LABioMed at Harbor-UCLA Med. Ctr.*

**Introduction:** Although leptin and insulin serve as hypothalamic modulators of appetite/satiety in the adult, they have critical neurotrophic properties during fetal life, including development of hypothalamic appetite pathways. Low birth weight (LBW) offspring have a programmed dysfunction in hypothalamic neuronal development with dysregulation of central signaling of orexigenic and anorexigenic (satiety) neuropeptides. As LBW offspring have decreased cord blood leptin and insulin levels, we hypothesized that reduced neurotrophic stimulation during critical periods may alter the development of appetite pathways. We utilized neural stem cells (NSC) to investigate the growth and differentiation of hypothalamic neuronal cells in response to leptin and insulin. We determined the putative signaling pathways essential for proliferation versus differentiation.

**Methods:** Hypothalamus from E20 control rat embryo and cultured in complete medium (CM) containing growth factors and heparin. At day 8-9 of culture, NSC were seeded in CM or differentiating medium (DM; without growth factors and heparin) for basal studies. For studies of neurotrophic proliferation responses, NSC cultured in CM were treated with leptin (10, 20, 40 ng/ml) or insulin (10, 20, 40 µg/ml) every 48h for 8 days and proliferation rate measured by MTT assay. For differentiation responses, NSC cultured in DM were treated with leptin or insulin (as above) and cell differentiation quantified by expression (Western Blot) of neuronal (NeuN, Tuj1) or astrocyte (GFAP) markers. Signaling pathways were examined by measure of select molecules (Notch 1, Hes1, pERK1/2 and pSTAT3) and NSC responses in the presence of selective pathway antagonists.

**Results:** Both leptin and insulin enhanced NSC *proliferation*. In CM cultures, there was a dose-dependent effect of leptin (35%, 39%, 72%) and insulin (23%, 28%, 43%) on NSC proliferation. However, there were selective NSC *differentiation* and signaling responses to leptin and insulin. Leptin treatment of NSC in DM cultures resulted in marked increase in expression of neuronal markers (NeuN: 185%; Tuj1: 46%) with a non-significant trend towards increased astrocyte marker (GFAP). In contrast, insulin treatment caused significant increase in GFAP (78%) with non-significant increment in NeuN and Tuj1. In studies of signaling responses, leptin and insulin induced NSC proliferation in association with increased Notch1 and Hes1, as well as increased phosphorylation of ERK1/2 and STAT3. In contrast, NSC differentiation was associated with inhibition of Notch1 and activation of ERK1/2 and STAT3 pathway. Inhibition of ERK activation by PD98059 or STAT3 by AG490 completely blocked leptin/insulin induced NSC proliferation and differentiation.

**Conclusions:** Leptin and insulin have potent effects on NSC proliferation, with selective effects on NSC differentiation, enhancing neuronal or glial cells, respectively. Both NSC proliferation and differentiation include ERK1/2 and STAT3 pathways, though only proliferation includes Notch1 and Hes1 signaling. These results indicate a critical role of in utero neural trophic factors and suggest that excess or deficient leptin/insulin associated with IUGR or macrosomic fetuses may permanently alter neural pathway development and adult behavior.

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presenting Author	Abstract	Session	Title
Bennett, Laura	Abstract 24	Session IV	The Role of Nitric Oxide in Vasodilation following Intrapleural OK-432 Administration in Preterm Fetal Sheep Departments of Physiology and Obstetrics and Gynaecology, The University of Auckland, New Zealand; Center for Perinatal Biology, Loma Linda University School of Medicine, Loma Linda, CA, USA
Blood, Arlin	Abstract 3	Session I	Blood Nitrite and Iron-Nitrosyl Hemoglobin Concentrations in Response to Acute Hypoxia in the Fetal Sheep Department of Pediatrics, Division of Neonatology; Center for Perinatal Biology, Loma Linda University Medical School, Loma Linda, CA
Booth, Lindsea	Abstract 36	Session VI	Maturation of the Generation and Control of Sympathetic Nerve Activity Department of Physiology, The University of Auckland, New Zealand
Drury, Paul	Abstract 48	Session VII	Cerebral Oxygenation and Epileptiform Activity after the Norwood Procedure for Hypoplastic Left Heart Syndrome Department of Physiology, University of Auckland, PICU, Starship Children's Hospital, Department of Paediatrics, University of Auckland, New Zealand
Feng, Xiaodi	Abstract 40	Poster	Small Increases in Fetal Cortisol Increase Expression of ENaC $\alpha$ in Fetal Lung without Changing Expression of Other ENaC Subunits or Aquaporins Department of Pharmacodynamics, University of Florida, Gainesville, FL USA
Fraser, Mhoyra	Abstract 41	Poster	Repeated exposure to TNF- $\alpha$ in an In Vitro Ovine Model of Preterm Infection/Inflammation-Mediated Brain Injury: Effects on MMP, TIMP and TACE expression The Liggins Institute, and Departments of Pharmacology and Physiology, University of Auckland, Auckland, New Zealand
Fraser, Mhoyra	Abstract 42	Poster	Repeated Systemic Fetal Administration Of LPS Upregulates The Expression of Tumour Necrosis Factor Alpha (TNF- $\alpha$ ) In The Periventricular White Matter Of The Preterm Ovine Fetus The Liggins Institute, and Physiology, University of Auckland, Auckland, New Zealand
Giussani, Dino	Abstract 11	Session II	Maternal Vitamin C Administration Improves Cognitive Function In Adulthood following Prenatal Hypoxia Department of Physiology Development & Neuroscience University of Cambridge, United Kingdom
Goyal, Ravi	Abstract 8	Session IV	Differential Role of $\alpha$ 1-Adrenergic Receptor Subtypes in Developing Cerebral Arteries Center for Perinatal Biology, Loma Linda University Medical School, Loma Linda, CA
Goyal, Ravi	Abstract 32	Session V	Antenatal Protein Malnutrition in the Mouse: Epigenetic Changes and

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Gray, Clint	Abstract 26	Session V	<p>Developmental Origin of Hypertension in Adult Center for Perinatal Biology, Loma Linda University Medical School, Loma Linda, CA</p> <p>Effects of a “Western” Diet on Maternal Metabolism and Fetal Development in Rats</p>
Green, Lucy	Abstract 31	Session V	<p>School of Veterinary Medicine and Science, Sutton Bonington, School of Human Development and School of Biosciences, Queens Medical Centre, University of Nottingham.</p> <p>Early life undernutrition in sheep induces sex- and tissue-specific effects on factors mediating insulin sensitivity and lipid handling</p>
Guissani, Dino	Abstract 30	Session V	<p>Institute of Developmental Sciences, Developmental Origins of Health and Disease Division, University of Southampton; Southampton General Hospital, Southampton; Department of Veterinary Reproduction, Royal Veterinary College, Hawkshead Lane, North Mymms</p> <p>Vitamins C and E ameliorate the programming of cardiac dysfunction in adult rats induced by neonatal dexamethasone treatment</p>
Guissani, Dino	Abstract 6	Session I	<p>Department of Physiology, Development and Neuroscience, University of Cambridge, U.K.; Department of Physiology, Anatomy and Genetics, University of Oxford, U.K.</p> <p>Maternal melatonin protects against the developmental programming of cardiovascular disease in hypoxic pregnancy</p>
Gunn, Alistair	Abstract 1	Session I	<p>Department of Physiology, Development and Neuroscience, University of Cambridge, U.K.; Department of Paediatrics, Maastricht University, The Netherlands</p> <p>Can insulin like growth factor-1 improve white matter protection with delayed cerebral hypothermia?</p>
Harding, Richard	Abstract 27	Session V	<p>Department of Physiology, The University of Auckland, New Zealand</p> <p>Episodic ethanol exposure has multiple effects on the fetus</p>
Herrera, E.A.	Abstract 22	Session IV	<p>School of Biomedical Sciences, Monash University, Melbourne Australia; Dept of OBGYN, University of Toronto, Canada; Dept of Pharmacol &amp; Toxicol, Queen’s University, Kingston, Canada; Dept of Anatomy and Cell Biology, University of Melbourne, Australia</p> <p>Antioxidant treatment prevents peripheral vascular dysfunction induced by neonatal glucocorticoids on weanling and adult rats</p>
Hirst, Jonathan	Abstract 54	Session VIII	<p>Department of Physiology, Development and Neuroscience, University of Cambridge, UK</p> <p>Suppression of 5<math>\alpha</math>-Reductase Isoforms by Betamethasone Treatment in the Placenta and Brain of Normal and Growth Restricted Fetal Guinea Pigs</p>
Jayawardene, Asanka	Abstract 15	Session III	<p>School of Biomedical Sciences &amp; Mothers and Babies Research Centre, University of Newcastle, Newcastle; Department of Physiology, Monash University, Melbourne, Australia</p> <p>Fetal Heart Rate (FHR) Variability in Obstetric Cholestasis (OC)</p>
			<p>Schools of Clinical Science and Electrical Systems and Applied Optics, University of Nottingham, UK.</p>

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Jenkin, Graham	Abstract 44	Poster	Melatonin administration in an ovine model of in utero infection - a prospect for neuroprotection? Department of Obstetrics & Gynaecology and Monash Immunology & Stem Cell Laboratories, Monash University, Clayton, Victoria Australia
Jenkin, Graham	Abstract 51	Session VII	Human amnion epithelial cells: potential clinical application Department of Obstetrics and Gynaecology, Monash Immunology and Stem Cell Laboratories, Monash University, Victoria, Australia
Jenkin, Graham	Abstract 52	Session VII	Isolation and characterization of ovine amniotic epithelial cells for use in pre-clinical trials Monash Immunology and Stem Cell Laboratories, Monash University, Clayton, Victoria, Australia
Jensen, Ellen	Abstract 53	Session VIII	Adenosine A1 Receptor Mediated Suppression of Adrenal Activity in Near-term Fetal Sheep Department of Physiology, Faculty of Medical and Health Sciences, and The Liggins Institute University of Auckland, Auckland, New Zealand; Center for Perinatal Biology, Loma Linda University School of Medicine, Loma Linda, CA
Kane, Andrew, D.	Abstract 10	Session II	Vitamin C prevents baroreflex and vasoconstrictor dysfunction in the adult rat induced by chronic fetal hypoxia Department of Physiology, Development and Neuroscience, University of Cambridge, UK
Kessler, Joerg	Abstract 47	Session VII	Development of venous liver perfusion in macrosomic non-diabetic fetuses University of Bergen, Department of Clinical Medicine, Bergen, Norway
Konishi, Shouhei	Abstract 20	Session IV	Effects of postnatal steroid administration on the rat model of chronic lung disease Maternal and Perinatal Care Center, Hokkaido University Hospital, Sapporo; Department of Perinatology, National cardiovascular center, Suita; Department of Veterinary Pathology, Obihiro University of Agricultural and Veterinary Medicine, Obihiro; Japan
Llanos, Anibal	Abstract 21	Session IV	Hemin Decreases Pulmonary Arterial Pressure in Hypertensive Newborn Lambs from the Andean Altiplano Laboratorios FFDD, Facultades de Medicina y; Ciencias Químicas-Farmacéuticas, INCAS, Universidad de Chile; Universidad de Tarapacá; Universidad Católica del Norte, Coquimbo, Chile; Universidad Cayetano Heredia, Lima, Perú; Department of ObGyn, University of California San Francisco, USA.
Makarenko, Irena	Abstract 35	Session VI	How each neuron of the mammillary body forms projections to thalamic and midbrain nuclei using collaterals of its axon. Institute of Developmental Biology RAS, Moscow, Russian Federation.
McCartney, Jarrett	Abstract 43	Poster	Aldosterone effects changes in lung compliance in the ovine fetal lung Departments of Pharmacodynamics and Physiology and Functional Genomics, University of Florida, Gainesville, FL USA

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Nechaeva, Marina	Abstract 13	Session II	Effect of acute hypoxia on the motor activity of the 10- and 14-day chick embryo Institute of Developmental Biology RAS, Moscow, Russia
Parer, Bill.	Abstract 50	Session VII	Standardized interpretation and management with a computerized decision-support tool in fetal heart rate monitoring Division of Maternal-Fetal Medicine, University of California San Francisco, CA; McGill University and LMS Medical Systems, Montreal, Canada
Pearce, William	Abstract 25	Session II	Does VEGF Mediate the Mitogenic Effects of Hypoxia in Large Arteries? Center for Perinatal Biology, Loma Linda University Medical School, Loma Linda, CA
Pretheeban, Manoja	Abstract 55	Session VIII	Identification and Quantitation of Phase I Hepatic Drug Metabolizing Enzymes in the Fetal, Newborn and Adult Sheep Department of Obstetrics and Gynecology, Child and Family Research Institute, Faculty of Medicine; Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, Canada
Probyn, Megan	Abstract 28	Session V	Effects of Moderate Prenatal Ethanol Exposure on Lactation, Mammary Gland Development and Pup Growth University of Queensland, Australia; The University of Melbourne, Australia; Monash University, Australia
Ratnayake, Udani	Abstract 9	Session II	The long term functional outcome of birth asphyxia in spiny mice Fetal and Neonatal Research Group, Department of Physiology, Monash University, Victoria, Australia
Reyes, Victor	Abstract 7	Session I	Blockade of soc and roc channels attenuates the hypoxia – induced pulmonary hypertension in vivo and small pulmonary arteries contractile status ex vivo Laboratorios FFDD y Bioquímica y Biología Molecular de la Hipoxia; bEscuela de Obstetricia, Facultades de Medicina y cCiencias Químicas-Farmacéuticas, dINCAS, Universidad de Chile; eUniversidad de Tarapacá, fUniversidad Católica del Norte, Chile; gUniversidad Cayetano Heredia, Perú; hDepartment of Physiology, Development & Neuroscience, University of Cambridge, UK
Richardson, Bryan	Abstract 4	Session I	Electrocortical (ECOG) activity in the ovine fetus with placental embolization and chronic hypoxemia Departments of Obstetrics and Gynecology, and Physiology and Pharamcology, University of Western Ontario, London, Ontario, Canada
Richardson, Bryan	Abstract 12	Session II	A fetal brain inflammatory response to repetitive umbilical cord occlusions (UCO) with worsening acidosis in the ovine fetus near term Departments of Obstetrics and Gynecology, and Physiology and Pharamcology, University of Western Ontario, London, Ontario, Canada

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Ross, Michael	Abstract 33	Session V	Enhanced adipose tissue desaturation activity promotes programmed obese phenotype in intrauterine growth restricted newborns Departments of Obstetrics and Gynecology and Pediatrics, LABioMed at Harbor-UCLA Medical Center, Los Angeles, CA
Ross, Michael	Abstract 37	Session VI	Developmental programming of dysfunctional hypothalamic neural stem cells in leptin deficient, low birth weight newborns Department of Obstetrics and Gynecology, LABioMed at Harbor-UCLA Medical Center, Los Angeles, CA
Ross, Michael	Abstract 39	Poster	Programmed enhanced adipogenesis contributes to adult obesity in growth restricted offspring: evidence from ex vivo adipose cell culture Department of Obstetrics and Gynecology, LABioMed at Harbor-UCLA Medical Center, Los Angeles, CA
Ross, Michael	Abstract 57	Session VIII	Rat embryonic hypothalamic neural stem cells (NSC) response to trophic factors: selective differentiation responses to leptin insulin Department of Obstetrics and Gynecology, LABioMed at Harbor-UCLA Medical Center, Los Angeles, CA
Schröder, Hobe J.	Abstract 49	Session VII	Magnetic resonance T2* measurements of oxygen saturation in blood samples of adult and fetal sheep, and of adult humans Center for Perinatal Biology, and Department of Radiology, Loma Linda University Medical School, Loma Linda, CA
Seron-Ferre, M	Abstract 56	Session VIII	In the fetal rat, the adrenal and heart contain a circadian clock Programa de Fisiopatología, ICBM, Facultad de Medicina, Universidad de Chile, Santiago; Women's Health, Arrowhead Regional Medical Center, Colton, CA; Universidad de Tarapacá, Arica, Chile.
Seron-Ferre, Maria	Abstract 29	Session V	Impact of Fetal Exposure to Maternal Melatonin on Amount and Functionality of Brown Adipose Tissue (BAT) in the Newborn. Programa de Fisiopatología, ICBM, Facultad de Medicina, Universidad de Chile, Santiago, Chile, Arrowhead Regional Medical Center, Colton Medical Center, Colton, California and Universidad de Tarapaca
Shizuko, Akiyama	Abstract 17	Session III	Designing the lighting environments of the neonatal intensive care unit Center for Perinatal Medicine, Departments of Obstetrics and Gynecology, Pediatrics, Tohoku University Hospital, Sendai; , Division of Neonatal Medicine, Miyagi Children's Hospital, Sendai; Atom Medical Corporation, Tokyo; Department of Perinatology, National Cardiovascular Center, Osaka; epartment of Cellular Signaling, Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai; epartment of Applied Biochemistry, Faculty of Agriculture, Utsunomiya University, Utsunomiya; Japan

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Ueda, Keiko	Abstract 14	Session III	<p>Fetal tachyarrhythmias: the Comparison between Cases with or without Intrauterine Treatment: A Retrospective Data Analysis from Japanese Population            Department of Perinatology, National Cardiovascular Center (NCVC), Osaka; Department of Pediatrics, Kurume University, Fukuoka; Department of Pediatric Cardiology, Maternal and Child of Osaka, Osaka, Department of Neonatology, Kanagawa Children's Hospital, Kanagawa, Japan; Department of Pediatric Cardiology, Saitama Medical University, Saitama, n5), Department of Cardiology, Shizuoka Children's Hospital, Shizuoka; Department of Pediatrics, Tsuba University, Ibaraki; Department of Obstetrics and Gynecology, Chiba; Department of Internal Medicine, NCVC, Osaka, Japan9), Department of Cardiology, Nagano Children's Hospital, Nagano; Department of Neonatology, Japanese Red Cross Medical Center, Tokyo; Department of Pediatric Cardiology, NCVC, Osaka; Department of Perinatology, National Child Health Center, Tokyo; Japan</p>
Walker, David	Abstract 2	Session I	<p>Maternal Creatine Supplementation During Pregnancy Protects the Newborn Spiny Mouse Brain from Intrapartum Hypoxia            Fetal &amp; Neonatal Research Group, Department of Physiology, Monash University, Clayton, Victoria; School of Exercise &amp; Nutrition Sciences, Deakin University, Burwood, Victoria, Australia</p>
Walker, David	Abstract 45	Poster	<p>Physiology of the ovary during pregnancy, parturition and lactation in the spiny mouse            University of Maastricht, Netherlands; Department of Physiology, Monash University, Clayton, Australia</p>
Walker, David	Abstract 46	Poster	<p>Adrenalectomy causes increased progenitor cell proliferation in the late gestation fetal sheep brain.            Fetal &amp; Neonatal Research Group, Department of Physiology, Monash University, Melbourne, Australia; Faculty of Medicine, University of Maastricht, Netherlands; Department of Pharmacology, University of Newcastle, Australia.</p>
Watanabe, Shinpei	Abstract 16	Session III	<p>Development of human photoreceptors            Center for Perinatal Medicine, Departments of Obstetrics and Gynecology, Pediatrics, Division of Neonatal Medicine, Tohoku University Hospital, Sendai; Department of Pediatrics, National Defense Medical College Hospital, Tokorozawa; Japan</p>
Wilson, Sean	Abstract 23	Session IV	<p>The Role of Calcium Activated Chloride Channels in Pulmonary Arterial Vasoconstriction is Influenced by Postnatal Maturity and Long-Term Hypoxic Stress            Division of Pulmonary and Critical Care, Center for Perinatal Biology, Loma Linda University School of Medicine, Dept. of Pharmacology, School of Medicine and School of Community Health Sciences, University of Nevada, Reno</p>
Wood, Charles	Abstract 58	Session VIII	<p>Department of Physiology and Functional Genomics, University of Florida, Gainesville, FL</p>

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Yawno, Tamara	Abstract 34	Session VI	<p><b>Behavioral Effects of Altering Pregnane Steroid Concentrations in The Brain of Normoxic and Asphyxiated Fetal Sheep</b>            Departments of Physiology and Obstetrics and Gynaecology, Monash University, Clayton Victoria; School of Biomedical Sciences, University of Newcastle, Callaghan NSW; National Trauma Research Institute, Alfred Hospital, Prahran , Victoria; Australia</p>
Yawno, Tamara	Abstract 5	Session I	<p><b>Maternal melatonin administration provides neuroprotection in late-gestation fetal sheep in response to umbilical cord occlusion</b>            Departments of Obstetrics and Gynaecology, Physiology, Monash Immunology and Stem Cell Laboratories, Monash University, Victoria, Australia</p>
Zimmerman, Luc	Abstract 18	Session III	<p><b>Early postnatal bronchoalveolar lavage fluid growth factor patterns and development of bronchopulmonary dysplasia</b>            Paediatrics and Statistics, Maastricht University Medical Centre, Maastricht, Netherlands; Neonatology, University Hospital Gasthuisberg, Leuven, Belgium</p>
Zimmerman, Luc	Abstract 19	Session III	<p><b>Antenatal steroids and neonatal outcome after chorioamnionitis in preterm infants: prospective cohort study and meta-analysis</b>            Paediatrics, Maastricht University Medical Centre, Maastricht, Netherlands; aediatrics, Obstetrics, and Pathology, Erasmus University Medical Centre, Rotterdam, Netherlands</p>

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