41st Annual Meeting

# Fetal and Neonatal Phisiological Society

August 31st - September 3rd, 2014
Saint Vincent - ITALY

**Organizing Committee**
Tullia Todros
Caterina Guiot
Enrico Bertino
Pietro Gaglioti
Alessandro Rolfo
Elena Olearo
University of Turin

**Venue**
Parc Hotel Billia
Saint Vincent, ITALY

# INDEX

# Welcome

The fifth decade of life is a wonderful one.
One has had enough experience and has already learned a lot; but there is still enough time ahead to plan the future with enthusiasm, to learn more, being more thoughtful.
Now our Society is just starting this exciting time in life.

The past forty years have been very special in the history of fetal and neonatal physiology. Technological advances have allowed, for the first time, the study of fetal growth and development in its natural environment, the uterus. This has stimulated the interest of obstetricians and neonatologists for fetal, placental and neonatal pathophysiology, thus implementing translational research.

To bring together basic science and clinical researchers has always been the value of our society. The other value is that the program of the meetings is based on sharing research progresses among all the members of the Society.

Our wish is that this year meeting, opening the fifth decade of FNPS life with an exciting program, comprising 64 oral presentations and 31 posters, stimulates young researchers to continue on the same track for at least the next forty years!

Sincerely,

***Tullia Todros***

& the Organizing Committee

***Caterina Guiot***

***Enrico Bertino***

***Pietro Gaglioti***

***Alessandro Rolfo***

***Elena Olearo***

# Fnps mission statement

The FNPS stimulates discussion and exchange of ideas between physiologists, obstetricians and neonatologists. The FNPS considers an informal gathering and presentations of new and preliminary data, especially by students and in-training investigators, essential to achieve goals.
The Society was founded in 1974 during an informal meeting in Oxford. Professor Geoffrey Dawes (1918-1996) and Dr. Gerhard (Bo) Gennser took the initiative and were made honorary members of the society in 1995.
The name of the annual conference (and Society) has changed several times, reflecting the widening scope of the society:
1974-80 Conference on Fetal Breathing
1981-83 International Conference on Fetal Breathing and other Movements
1984-95 Society for the Study of Fetal Physiology
1996-present Fetal and Neonatal Physiological Society
Over the years the Society has maintained its informal character and a lack of rigid structures. Those who have attended at least one of the previous three meetings are members of the Society and will be informed about the next meeting. Abstracts for the Annual Meeting are requested two months before the meeting and are compiled in the Book of Abstracts to encourage recent and preliminary data to be presented.
The Organizational Coordinator will be selected by the Organizational Committee and shall serve the three years. The Organizational Committee shall consist of representatives from Africa, Asia, Australia, Canada, continental Europe, South America, the United Kingdom and the United States of America and shall be selected by the committee.
The Annual Meeting will be held in Europe, North America and the Southern Hemisphere, in June-September, as determined by the Organizational Committee. Approximately half of the meetings will be held in Europe.
Any residual funds from the prior meeting shall be passed on to the coordinator for the next meeting. Audit will not be required if the residual funds are less than 10,000 USD. The (local) Organizing Committee shall have the right to solicit funds in the name of the Society from organizations for the purpose of providing financial support for students and fellow-in-training to attend the meeting of the Society.

To see more information about the society visit the web site
*http://www.fnps-society.org/index.html*





# Fnps previous meetings

1974 Oxford, United Kingdom
1975 Oxford, United Kingdom
1976 Malmö, Sweden
1977 Oxford, United Kingdom
1978 Nijmegen, The Netherlands
1979 Paris, France
1980 Oxford, United Kingdom
1981 Maastricht, The Netherlands
1982 London, Canada
1983 Malmö, Sweden
1984 Oxford, United Kingdom
1985 Haifa, Israel
1986 Banff, Canada
1987 Groningen, The Netherlands
1988 Cairns, Australia
1989 Reading, United Kingdom
1990 Pacific Grove, USA
1991 De Eemhof, The Netherlands
1992 Niagara-the-Lake, Canada
1993 Plymouth, United Kingdom
1994 Palm Cove, Australia
1995 Malmö, Sweden
1996 Arica, Chile
1997 S.Margherita Ligure, Italy
1998 Lake Arrowhead, USA
1999 Vlieland, The Netherlands
2000 Southampton, United Kingdom
2001 Auckland, New Zealand
2002 Prague, Czech Republic
2003 Banff, Canada
2004 Tuscany, Italy
2005 Glenelg, South Australia
2006 Cambridge, United Kingdom
2007 Senday, Japan
2008 Maastricht, The Netherlands
2009 Lake Arrowhead, USA.
2010 Winchester, United Kingdom
2011 Palm, Cove, Australia
2012 Utrecht, The Netherlands
2013 Puerto Varas, Chile

# Fnps board members 2014

Jan Nijhuis (The Netherlands, Immediate Past President)
Laura Bennet (New Zealand)
Dino Giussani (UK, Scribe; President Elect)
Lucy Green (UK)
Emilio Herrera (Chile, Scribe Elect)
Tomoaki Ikeda (Japan)
Brian Koos (USA)
Carina Mallard (Sweden)
Tim Moss (Australia)
Julian Parer (USA)
Donald Peebles (UK)
Dan Rurak (Canada)
Charles Wood (USA)
Luc Zimmermann (The Netherlands)





# Minutes of the 40th FNPS Annual General Meeting

Puerto Varas, Chile • 1-4 September 2013

Present: Laura Bennet, Dino Giussani (Scribe), Emilio Herrera, Tomoaki Ikeda, Graham Jenkin (guest), Jan Nijhuis (Chair), Bill Parer, Dan Rurak, Tullia Todros (guest), Claudia Torres Farfan (guest), Charles Wood, Luc Zimmermann.

1. **Professor David Barker CBE FRS In Memoriam.**
   There was one minute silence for the Society to pay respect to David Barker, who died on 27th August 2013.
2. **Meeting minutes.**
   The minutes of the 39th Annual FNPS meeting at Utrecht, The Netherlands 2012, were accepted after inclusion of Dan Rurak on the attendance list to the Board Meeting.
3. **Vote of thanks.**
   Jan Nijhuis, as President, expressed a vote of thanks to the Local Organising Committee and Sponsors for an excellent meeting.
4. **Elections.**
   Dino Giussani was elected as the next President of the Society and Emilio Herrera would be the next FNPS Scribe. Both offices would start at the next FNPS meeting.
5. **FNPS board membership.**
   Some members of the Society expressed an interest in becoming members of the FNPS board. Their expression of interest was discussed and it was decided to invite applications only when positions became vacant. Possible candidate names were noted for future board openings. The board also agreed that it would be a good idea to have a Junior Member Representative on the board.
6. **FNPS membership**.
   The board discussed ideas to increase the membership of the Society. It was felt that a junior group promoting membership to the Society would be a good idea. Laura Bennet to investigate.
7. **Meeting attendance by board members.**
   The meeting agreed that if any board member did not attend the FNPS meeting during three consecutive years, then they would be asked to step down from the board. The incoming President would investigate this.
8. **Paediatric representation.**
   The board felt that it would be a good idea to make a special effort to engage greater representation from paediatrics at the meeting. The European Workshop on Neonatal Transition at Prato next year organised by The Ritchie Centre would provide a good opportunity to do this.
9. **Aspen Perinatal Conference.**
   The meeting was made aware of this meeting that occurs every 3 years. The next one will be in 2016 and will be organised by Tim Regnault Dovetailing future meeting with this meeting or other relevant meetings such as IFPA was discussed.
10. **Future meetings:**
    (i) 2014. St Vincent, Italy. Tullia Todros agreed to host meeting. This FNPS meeting is to be preceded by the Satellite Workshop at Prato, organised by Graham Jenkin. Graham Jenkin to investigate synergistic possibilities with Tullia Todros.
    (ii) 2015. Canada or Japan proposed.

(iii) 2016. Possibility of Cambridge, perhaps to coincide with the centenary celebration of some of Joseph Barcroft's initial findings on Fetal Physiology.

11. **Sporting event.**
The FNPS board restated the point that mixing up the teams with individuals from different groups and countries is a positive move to encourage social integration. Future organisers should take this into consideration.

12. **Last session.**
The board restated that in order to facilitate the award of student prizes, the FNPS board recommended that no student should be placed in the last scientific session of the meeting.

13. **Prizes:**
*FNPS 2013 Prize* Heather L. Blackmore
Programming of cardiac dysfunction by maternal diet-induced obesity.
University of Cambridge

*FNPS 2013 Prize* Lotte van den Heuij
The effects of human amniotic epithelial stem cells on evolving brain injury in preterm fetal sheep.
University of Auckland

*FNPS 2013 Prize* Stacey J. Ellery
Creatine Supplementation Protects the Neonatal Spiny Mouse Following Birth Asphyxia, but does it affect the Mother?
Monash University

*Anillo Prize* Nozomi Itani
Melatonin rescues endothelial dysfunction during hypoxic development in the chick embryo
University of Cambridge

*Anillo Prize* Fernanda Amaral
Absence of maternal melatonin is detrimental to melatonin synthesis in adult offspring
University of Sao Paulo

*Anillo Prize* Pamela Alonso-Vazquez
Persistent down-regulation of KChiP2 may contribute to the adult cardiac hypertrophy enforced by gestational chronodisruption.
Universidad Austral de Chile

*Bo Gennser Memorial Prize (Poster)* Alejandro González-Candia
Prenatal melatonin improves systemic and cerebrovascular function in neonatal lambs gestated under chronic hypoxia.
Universidad de Chile

*Tania Gunn Memorial Prize (Postdoc)* Emily J. Camm
Chronic prenatal hypoxia in the rat affects cognitive function and brain structure in adulthood: intervention by vitamin C.
University of Cambridge

*Tania Gun Memorial Prize* (Trainee) James Aridas
Melatonin treatment of acutely asphyxiated newborn lambs as a potential adjuvant therapy to hypothermia.
Monash University

Respectfully submitted,

***Dino A. Giussani***
*FNPS Scribe*





# Awards

**YOUNG INVESTIGATOR SAMSUNG AWARDS**
There will be two prizes kindly offered by Samsung, one for the Best Oral Presentation (500€) and one for the Best Poster (500€), to support Young Investigators in the field of Fetal & Neonatal Medicine.
Young Investigators are defined as students or researchers in full-time training under the age of 40.

Awards Committee
President: Tim Moss
Members: Tullia Todros, Dino Giussani

**TANIA GUNN MEMORIAL PRIZE**
This prize was introduced in FNPS 2011 in memory of Tania Gunn (1932-1999), professor of Neonatology at Auckland, New Zealand. She is remembered for her important studies of the control of thermoregulation at birth and safety of therapeutic hypothermia for babies with acute encephalopathy

**BO GENNSER MEMORIAL PRIZE (POSTER)**
This prize was introduced in last year FNPS meeting in memory of Bo Gennser (1929-2010), professor in Obstetrics & Gynecology at Lund University, Sweden. He is recognized for developing new methods in recording fetal breathing movements and the evaluation of fetal real-time ultrasound. In 1974, he and Geoffrey Dawes initiated the Fetal Breathing Conferences, that later end up in the Fetal & Neonatal Physiological Society

## With the support of

Regione Valle D'Aosta

Università degli Studi di Torino

**AGUI**
Associazione Ginecologi Universitari Italiani

## Thanks to

Alfa Wassermann SPA

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Hologic Italia SPA

Istituto Ganassini SPA

Samsung Electronics Italia SPA





## Program at a glance

### August 31st, 2014 • PRE MEETING AFTERNOON

6.00 pm Registration

7.00 pm Annual Geoffrey Dawes Lecture - Maria Lodovica Gullino (University of Turin - Italy)

***Chair: Tullia Todros*** *(University of Turin - Italy)*

8.00 pm *Welcome Dinner*

### September 1st, 2014 • MEETING

8.00 - 8.30 am Registration

8.30 - 10.30 am ORAL SESSION I - FETAL & NEONATAL CARDIOVASCULAR (I)

***Chairs: Dino Giussani*** *(University of Cambridge - UK)* ***& Luc Zimmermann*** *(Maastricht University - Netherlands)*

10.30 - 11.00 am *Coffee Break + Posters viewing*

11.00 - 1.00 pm ORAL SESSION II - DEVELOPMENTAL ORIGINS OF HEALTH AND DISEASE

***Chairs: Laura Bennet*** *(University of Auckland, New Zealand)* ***& Donald Peebles*** *(University College London, UK)*

1.00 - 2.30 pm *Lunch*

2.30 - 4.30 pm ORAL SESSION III - PLACENTA & MISCELLANEOUS

***Chairs: Claire Roberts*** *(University of Adelaide, Australia)* ***& Chiara Mandò*** *(University of Milan, Italy)*

4.30 - 5.00 pm *Coffee Break + Posters viewing*

5.00 - 7.00 pm ORAL SESSION IV - FETAL & NEONATAL BRAIN (I)

***Chairs: Graham Jenkin*** *(Monash University, Australia)* ***& Emilio Herrera*** *(University of Chile, Chile)*

8.00 pm *Dinner*

## September 2nd, 2014 • MEETING

8.30 - 10.30 am ORAL SESSION V - MISCELLANEOUS

***Chairs: Tomoaki Ikeda*** *(Mie University Graduate School of Medicine, Japan)* ***& Alessandro Rolfo*** *(University of Turin, Italy)*

10.30 - 11.00 am *Coffee Break + Posters viewing*

11.00 - 1.00 pm ORAL SESSION VI - FETAL & NEONATAL BRAIN (II)

***Chairs: Tullia Todros*** *(University of Turin, Italy)* ***& Dan Rurak*** *(University of British Columbia, Canada)*

1.00 - 2.30 pm *Lunch*

2.30 - 3.30 pm POSTERS PRESENTATION

8.00 pm *Dinner*

## September 3rd, 2014 • MEETING

8.30 - 10.30 am ORAL SESSION VII - FETAL & NEONATAL CARDIOVASCULAR (II)

***Chairs: Julian T. Parer*** *(University of California - USA)* ***& Jan Nijhuis*** *(Maastricht University - Netherlands)*

10.30 - 11.00 am *Coffee Break*

11.00 - 1.00 pm ORAL SESSION VIII - NUTRITION AND GROWTH & TRANSLATIONAL STUDIES

***Chairs: Tim Moss*** *(Monash University, Australia)* ***& Jan Derks*** *(University Medical Center -Utrecht, Netherlands)*

1.00 pm *Closing Remarks and Awards Presentation*

***Awards Committee***
*President: Tim Moss*
*Members: Tullia Todros, Dino Giussani*

1.30 pm *Lunch*





# Program

## August 31st, 2014 • PRE MEETING AFTERNOON

6.00 pm Registration

7.00 pm Annual Geoffrey Dawes Lecture - ***Maria Lodovica Gullino*** *(University of Turin - Italy)*

***Chair: Tullia Todros*** *(University of Turin - Italy)*

8.00 pm *Welcome dinner*

## September 1st, 2014 • MEETING

8.00 - 8.30 am Registration

8.30 - 10.30 am ORAL SESSION I - FETAL & NEONATAL CARDIOVASCULAR (I)

***Chairs: Dino Giussani*** *(University of Cambridge - UK)* ***& Luc Zimmermann*** *(Maastricht University - Netherlands)*

8.30 - 8.45 *O1 -* ***Adenosine A2 receptors and nitric oxide-dependent signaling pathway may be involved in fetal endothelial cells proliferation during gestational diabetes***
C. Escudero - Universidad del Bio Bio, Chillan - Chile

8.45 - 9.00 *O2 -* ***Chronic hypoxia in the growth-restricted fetus is associated with FHR overshoot during brief repeated umbilical cord occlusions in near-term fetal sheep***
G. Wassink - Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand

9.00 - 9.15 *O3 -* ***Altered Cardiopulmonary And Cerebral Hemodynamic Responsiveness To Early Inhaled Nitric Oxide In Growth Restricted Preterm Lambs***
G. Polglase - The Ritchie Centre, Monash University, Melbourne, Australia

9.15 - 9.30 *O4 -* ***Foetal and umbilical vascular reactivity in a model of IUGR through gradual uterine artery occlusion in guinea pigs***
B.J. Krause - Pontificia Universidad Catolica de Chile, Santiago - Chile

9.30 - 9.45 *O5 -* ***The mitochondrial targeted antioxidant MitoQ and Sildenafil rescue endothelial dysfunction in the hypoxic chick embryo***

K.L. Skeffington - Department of Physiology, Development and Neuroscience, Cambridge University, Cambridge, United Kingdom

9.45 - 10.00 *O6* - ***The development of primary hemostasis and platelet function during mouse fetal life in vivo***
M. Sperandio - Walter Brendel Center of Experimenal Medicine (WBex), Ludwig Maximilians University (LMU), Munich, Germany

10.00 - 10.15 *O7* - ***Vasoactive effects of serotonin in the ductus arteriosus of the chicken embryo***
E. Villamor - Department of Pediatrics, School for Oncology and Developmental Biology (GROW), Maastricht University Medical Center (MUMC+), Maastricht, Netherlands

10.15 - 10.30 *O8* - ***Doppler blood flow variables in the human fetal middle cerebral (MCA), right pulmonary (RPA) and umbilical arteries (UA): evidence for fluctuating arterial Po2***
D. Rurak - Child & Family Research Instiute, University of British Columbia, Vancouver, Canada

10.30 - 11.00 am *Coffee Break + Posters viewing*

11.00 - 1.00 pm ORAL SESSION II - DEVELOPMENTAL ORIGINS OF HEALTH AND DISEASE

***Chairs: Laura Bennet*** *(University of Auckland, New Zealand)* ***& Donald Peebles*** *(University College London, UK)*

11.00 - 11.15 *O9* - ***Growth and endothelial function in the first 2 years of life: a longitudinal study***
L. Zimmermann - Pediatrics MUMC, Maastricht - Netherlands

11.15 - 11.30 *O10* - ***Cardiac structural and functional changes in fetal and adult offspring of hypoxic pregnancy can be prevented with maternal antioxidant treatment***
E.I. Camm - University of Cambridge, Cambridge - United Kingdom

11.30 - 11.45 *O11* - ***Metabolic and morphological changes during obesity with and without pregnancy***
A. Sirico - Università Federico II, Napoli - Italy

11.45 - 12.00 *O12* - ***Sensitivity analysis for validation of a model to simulate fetal heart rate decelerations during labor***
L. Bullens - Maastricht University Medical Center, Department of Obstetrics and Gynecology, Maastricht University Medical Center, Maastricht, Netherlands

12.00 - 12.15 *O13* - ***Intergenerational Programming of Heart Disease and Heritability of Cardioprotection via the Maternal Mitochondria***
D. Giussani - Department of Physiology, Development & Neuroscience, University of Cambridge, Cambridge, United Kingdom

12.15 - 12.30 *O14* - ***Maternal iron status in early pregnancy and arterial stiffness in infants: an observational study***





N. Alwan - University of Leeds, Nutritional Epidemiology Group, Leeds, United Kingdom

12.30 - 12.45 *O15* - ***Induced prematurity in the guinea pig; a new model to study the life-course effects of preterm birth***
M. Berry - Department of Paediatrics & Child Health, University of Otago, Wellington, Wellington, New Zealand

12.45 - 1.00 pm *O16* - ***Risk of respiratory morbidity and mode of delivery in late preterm infants***
G. Simonazzi - Department of Medical Surgical Sciences, Division of Obstetrics and Prenatal Medicine, Sant'Orsola-Malpighi Hospital, University of Bologna, Bologna, Italy

1.00 - 2.30 pm *Lunch*

2.30 - 4.30 pm ORAL SESSION III - PLACENTA & MISCELLANEOUS

***Chairs: Claire Roberts*** *(University of Adelaide, Australia)* ***& Chiara Mandò*** *(University of Milan, Italy)*

2.30 - 2.45 *O17* - ***Elevated endothelial cell markers and tyrosine 1175 phosphorylation of vascular endothelial growth factor receptor type 2 in placentas from gestational diabetes***
C. Escudero - Universidad del Bio Bio, Chillan - Chile

2.45 - 3.00 *O18* - ***Over-expression of tumor-suppressor genes LDOC1, PARP1 and Caspase3 in Preeclamptic (PE) Placenta-derived Mesenchymal Stromal Cells (PDMSCs)***
A.M. Nuzzo- University of Turin, Turin - Italy

3.00 - 3.15 *O19* - ***Human amnion epithelial cells mediate lung repair by influencing macrophage and regulatory T cell response***
R. Lim - The Ritchie Centre, Monash Institute of Medical Research, Clayton, Australia

3.15 - 3.30 *O20* - ***Placental Telomere Shortening in Stillbirth: A Sign of Premature Senescence?***
F. Ferrari - University Hospital of Modena, Modena - Italy

3.30 - 3.45 *O21* - ***Inflammation-induced surfactant protein A expression is mediated by pulmonary fibroblasts***
A. McDougall - The Ritchie Centre, MIMR-PHI institute and The Department of Biochemistry and Molecular Biology, Monash Medical Centre and Monash University, Melbourne, Australia

3.45 - 4.00 *O22* - ***Effect of hypothyroidism on pancreatic β-cell mass and circulating insulin concentration in ovine fetuses***
S.E. Harris - Oxford Brookes University, Oxford, United Kingdom

4.00 - 4.15 *O23* - ***Exhaled CO2 measurement in ventilated infants***
K. Suzuki - Department of Pediatrics, Tokai University School of Medicine, Isehara, Japan

4.15 - 4.30 *O24* - ***Controlling contractions in human myometrium, an eye on channels***
H. Parkington - Department of Physiology, Monash University, Melbourne, Australia

4.30 - 5.00 pm *Coffee Break + Posters viewing*

5.00 - 7.00 pm ORAL SESSION IV - FETAL & NEONATAL BRAIN (I)

***Chairs: Graham Jenkin*** *(Monash University, Australia)* ***& Emilio Herrera*** *(University of Chile, Chile)*

5.00 - 5.15 *O25* - ***Synergistic white matter protection with acute-on-chronic endotoxin and subsequent asphyxia in preterm fetal sheep***
L. Bennet - Department of Physiology, The University of Auckland, Auckland, New Zealand

5.15 - 5.30 *O26* - ***The effect of customized versus population standards on the identification of small for gestational age in a cohort of neonates who had intra-partum acute-distress***
V. Seravalli - University of Florence, Florence - Italy

5.30 - 5.45 *O27* - ***Peripheral Myeloid Cells Invade the Brain Following Neonatal Hypoxia-Ischemia***
C. Mallard - Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

5.45 - 6.00 *O28* - ***Perinatal asphyxia leads to region specific overexpression of PARP-1, increasing the expression of pro-inflammatory cytokines and cell death: prevention by systemic nicotinamide treatment***
M. Herrera-Marschitz - Medical Faculty, ICBM, University of Chile, Santiago, Chile

6.00 - 6.15 *O29* - ***Status Epilepticus after Prolonged Umbilical Cord Occlusion Is Associated with Greater Neural Injury Fetal Sheep at Term-Equivalent***
P. Drury - Department of Physiology, University of Auckland, Auckland, New Zealand

6.15 - 6.30 *O30* - ***Suppression of neurosteroid synthesis has adverse long-term effects on outcomes***
J. Hirst - Hunter Medical Research Institute, School of Biomedical Sciences and Pharmacy, University of Newcaslte, Callaghan, Australia

6.30 - 6.45 *O31* - ***The impact of antenatal steroids and magnesium sulphate on neurotrophic factors in preterm infants***
N. Hodyl - Robinson Research Institute & School of Paediatrics and and Reproductive Health, University of Adelaide, Adelaide, Australia

6.45 - 7.00 *O32* - ***Progesterone treatment of the growth restricted fetus - a natural therapy***
T. Yawno - The Ritchie Centre, MIMR-PHI - Australia

8.00 pm *Dinner*





## September 2nd, 2014 • MEETING

8.30 - 10.30 am ORAL SESSION V - MISCELLANEOUS

***Chairs: Tomoaki Ikeda*** *(Mie University Graduate School of Medicine, Japan)* ***& Alessandro Rolfo*** *(University of Turin, Italy)*

8.30 - 8.45 *O33* - ***The effect of Holder pasteurization on human milk glycosaminoglycans***
C. Peila - University of Turin, Turin - Italy

8.45 - 9.00 *O34* - ***Low Molecular Weight Heparin Modulates D6 Decoy Receptor Expression in Preeclamptic Human Umbilical Vein Endothelial Cells***
R. Barrile - University of Turin, Turin - Italy

9.00 - 9.15 *O35* - ***Influence of human amnion epithelial cells on the fetal inflammatory response***
T. Moss - The Ritchie Centre & Department of Obstetrics and Gynaecology, MIMR-PHI Institute of Medical Research & Monash University, Melbourne, Australia

9.15 - 9.30 *O36* - ***Effects of Prenatal Hypoxia on Cardiac Function over Ageing***
Y. Niu - University of Cambridge, Cambridge - United Kingdom

9.30 - 9.45 *O37* - ***Do deficits in Trop2 lead to reduced cerebellar granule cell migration in growth-restricted offspring?***
A. McDougall - The Ritchie Centre, MIMR-PHI institute and The Department of Biochemistry and Molecular Biology, Monash Medical Centre and Monash University, Melbourne, Australia

9.45 - 10.00 *O38* - ***Differences in heart structure of lambs immediately after preterm and term birth***
V. Nguyen - Department of Anatomy and Developmental Biology, Monash University, Clayton, Australia

10.00 - 10.15 *O39* - ***Human amniotic epithelial cells: chronic effects on evolving brain injury in preterm fetal sheep***
L. van den Heuij - Fetal Physiology and Neuroscience Group, Dept Physiology, University of Auckland, Auckland, New Zealand

10.15 - 10.30 *O40* - ***The effect of increased placental vascular resistance and hypoxemia on fetal left ventricular myocardial performance index***
A. Bhide - Fetal Medicine Unt, St. George's Hospital, St George's, University of London, London, United Kingdom

10.30 - 11.00 am *Coffee Break + Posters viewing*

11.00 - 1.00 pm ORAL SESSION VI - FETAL & NEONATAL BRAIN (II)

***Chairs: Tullia Todros*** *(University of Turin, Italy)* ***& Dan Rurak*** *(University of British Columbia, Canada)*

11.00 - 11.15 *O41* - ***Cerebral Oxygenation Deficits are assocaited with Persistent Periodic Breathing in Preterm Infants After Hospital Discharge***

R. Horne - The Ritchie Centre MIMR-PHI Institute of Medical Research, Monash University, Melbourne, Australia

11.15 - 11.30 *O42* - ***Exploring umbilical cord blood stem cell populations for the prevention of cerebral palsy***
C. McDonald - The Ritchie Centre, MIMR-PHI institute, Clayton, Australia

11.30 - 11.45 *O43* - ***Effects of antenatal magnesium sulphate treatment for neonatal neuro-protection on cerebral oxygen kinetics***
M. Stark - Robinson Research Institute & School of Paediatrics and and Reproductive Helath, University of Adelaide, Adelaide, Australia

11.45 - 12.00 *O44* - ***Prediction of hypothermic neuroprotection with EEG monitoring after asphyxia in preterm fetal sheep***
G. Wassink - Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand

12.00 - 12.15 *O45* - ***Unmet oxygen demand, critical hypoxic ischaemia and brain injury in preterm newborns***
M. Stark - Robinson Research Institute & School of Paediatrics and and Reproductive Health, University of Adelaide, Adelaide, Australia

12.15 - 12.30 *O46* - ***Daily high-dose caffeine administration disrupts development of Purkinje cells in the fetal ovine cerebellum***
M. Tolcos - The Ritchie Centre, MIMR-PHI Institute for Medical Research, Melbourne, Australia

12.30 - 12.45 *O47* - ***Is a longer better? Neuroprotection with delayed selective head cooling for three or five days after global cerebral ischaemia in the near-term fetal sheep***
J. Davidson - The University of Auckland, University, Auckland, New Zealand

12.45 - 1.00 *O48* - ***Chronic high-dose caffeine exposure injures the developing white matter in the ovine fetus***
R. De Matteo - Department of Anatomy and Developmental Biology, Monash University, Melbourne, Australia

1.00 - 2.30 pm *Lunch*

2.30 - 3.30 pm POSTERS PRESENTATION

P1 - *Type 1 Cannabinoid Receptors Blockade Simultaneous to Nociceptive Stress During Lactation, Perturb the Hepatic Endocannabinoid System Leading to Lipid Accumulation in Liver of Adult Mice*

P2 - *Maternal Body Mass Index influences Umbilical Artery Doppler Velocimetry in physiologic pregnancies*

P3 - *Connexin hemichannel blockade after fetal cerebral hypoxia-ischaemia improves survival of striatal phenotypic neurons*





P4 - *Spontaneous pre-existing hypoxia does not affect brain damage after global cerebral ischaemia in the near-term fetal sheep*

P5 - *Neurotrophin levels across gestation and in infants born small for gestational age*

P6 - *Progesterone replacement therapy following preterm birth increases circulating cortisol in male neonates*

P7 - *Neuroprotective effects of melatonin in hypoxic-ischemic brain injury of preterm infants*

P8 - *Antenatal Azithromycin Treatment for Intra-amniotic Ureaplasma Infection Improves. Neurobehavioral and Cognitive Development in Prematurely Born Rhesus Macaques.*

P9 - *Pre- and post- natal effects of melatonin on pulmonary prostanoids function in chronic hypoxic lambs*

P10 - *PDGF expression and pulmonary artery remodeling in newborn lambs differentially exposed to perinatal hypoxia.*

P11 - *Pulmonary anti-remodeling effects of melatonin on chronic hypoxic newborn sheep with pulmonary hypertension.*

P12 - *A case of fetal parvovirus B19 myocarditis that caused terminal heart failur*

P13 - *Reduction of cardiac oxygen consumption during fetal heart rate decelerations: investigations with a simulation model*

P14 - *Age-related changes and effects of mild hypothermia on carotid artery reactivity in newborn rats*

P15 - *Protease-activated receptor (PAR)-mediated contraction of the chicken ductus arteriosus*

P16 - *Investigating the mechanisms underlying bronchopulmonary dysplasia using an in vitro cell stretch model*

P17 - *Effects of Holder pasteurization on the protein profile of human milk*

P18 - *Is Umbilical Artery Dopplervelocimetry a predictor of Feeding Intolerance in preterm newborns?*

P19 - *Oxygen-loaded nanodroplets counteract hypoxia effects on MMP/TIMP balances in human placental chorionic villi*

P20- *Characterization of human placenta-derived stem cells in intrauterine growth restriction*

P21- *Effect of Bisphenol A on endometrial and placental physiology*

P22 - *Low Molecular Weight Heparin (LMWH) Inhibits Placenta Inflammation by Tuning HMGB1's Affinity for RAGE Receptor*

P23 - *Gestational Diabetes Mellitus (GDM) Modulates Human Placental Apoptosis*

P24 - *Fine particulate matter in the air within the limits recommended by WHO alters placental structure and circulating and local Renin Angiotensin System*

P25 - *Folate transporters expression in placentas related to birth weight and concentrations of folates in cord blood.*

P26 - *Evidences that high- or low-salt intake during pregnancy does not alter kidney structure in the newborns*

P27 - *Changes in fetal heart rate patterns in preterm prelabor rupture of membranes with and without histological chorioamnionitis*

P28 - *The Perinatologists' Family Tree - Reloaded*

P29 - *Do SGA fetuses have less reserves in labour than AGA fetuses? A retrospective study*

P30 - *The role of perinatal medical center for treating HIV-infected pregnant women with premature delivery in Japan*

P31 - *Predictive factors for Mirror Syndrome and evaluation of neonatal outcomes in a population of fetuses with effusion*

8.00 pm *Dinner*

## September 3rd, 2014 • MEETING

8.30 - 10.30 am ORAL SESSION VII - FETAL & NEONATAL CARDIOVASCULAR (II)

***Chairs: Julian T. Parer*** *(University of California - USA)* ***& Jan Nijhuis*** *(Maastricht University - Netherlands)*

8.30 - 8.45 am *O49* - ***Does fetal growth restriction alter the cardiopulmonary and cerebral hemodynamic response to early surfactant therapy?***
A. Malhotra - Monash Newborn, Monash University, Melbourne, Australia

8.45 - 9.00 *O50* - ***Pravastatin rescues cardiac dysfunction during hypoxic development in the chick embryo***
N. Itani - University of Cambridge, Cambridge - United Kingdom

9.00 - 9.15 *O51* - ***The effect of magnesium sulphate on the preterm fetal cardiovascular and cerebrovascular esponses to profound asphyxia***
*R. Galinsky - University of Auckland, Auckland - New Zealand*

9.15 - 9.30 *O52* - ***Pulmonary antioxidant capacity and oxidative stress in chronic***





***hypoxic lambs treated with prenatal melatonin***
E. Herrera - Pathophysiology Program, Faculty of Medicine; Universidad de Chile, Santiago, Chile

9.30 - 9.45 *O53* - ***Platelet counts in the first seven days of life and patent ductus arteriosus in preterm very low birth weight infants***
E. Villamor - Department of Pediatrics, School for Oncology and Developmental Biology (GROW), Maastricht University Medical Center (MUMC+), Maastricht, Netherlands

9.45 - 10.00 *O54* - ***Computer simulation of the cardiotocogram: the effect of catecholamines***
G. Jongen - Eindhoven University of Technology, University, Eindhoven, Netherlands

10.00 - 10.15 *O55* - ***Influence of gravity on umbilical blood flow and blood distribution during delayed umbilical cord clamping at birth***
K. Crossley - The Ritchie Centre & Department of Obstetrics and Gynaecology, Monash Institute of Medical Research-Prince Henry's Institute & Monash University, Clayton, Australia

10.15 - 10.30 *O56* - ***Repeated acute on chronic lipopolysaccharide exposure is associated with suppression of fetal heart rate variability in preterm fetal sheep***
C. Lear - Fetal Physiology and Neuroscience Group, University of Auckland, Auckland, New Zealand

10.30 - 11.00 am *Coffee Break*

11.00 - 1.00 pm ORAL SESSION VIII - NUTRITION AND GROWTH & TRANSLATIONAL STUDIES

***Chairs: Tim Moss*** *(Monash University, Australia)* ***& Jan Derks*** *(University Medical Center -Utrecht, Netherlands*

11.00 - 11.15 *O57* - ***Cytomegalovirus during pregnancy: outcomes of non primary versus primary maternal infections***
G. Simonazzi - Department of Medical Surgical Sciences, Division of Obstetrics and Prenatal Medicine, Sant'Orsola-Malpighi Hospital, University of Bologna, Bologna, Italy

11.15 - 11.30 *O58* - ***Amnion cells activate endogenous lung progenitors to augment lung repair***
R. Lim - The Ritchie Centre, Monash Institute of Medical Research, Clayton, Australia

11.30 - 11.45 *O59* - ***Reduced uterine blood flow induces asymmetric growth restriction in spiny mouse fetuses***
H. Dickinson - The Ritchie Centre, MIMR-PHI Institute, Melbourne - Australia

11.45 - 12.00 *O60* - ***The effects of obesity on hippocampal function in the rat***
*H. Coleman - Department of Physiology, Monash University, Melbourne, Australia*

12.00 - 12.15 *O61* - ***Maintenance of human amnion epithelial cell phenotype in***

***pulmonary surfactant: A potential novel combination therapy.***
C. McDonald - The Ritchie Centre, MIMR-PHI institute, Clayton, Australia

12.15 - 12.30 *O62* - ***A new 3 tiered approach predicts risk for major pregnancy complications***
C. Roberts - Robinson Research Institute, University of Adelaide, Adelaide, Australia

12.30 - 12.45 *O63* - ***Lung aeration at birth in a rabbit model of congenital diaphragmatic hernia***
M. Wallace - The Ritchie Centre, MIMR-PHI Institute, Dept of Obstetrics and Gynecology, Monash University, Melbourne, Australia

12.45 - 1.00 *O64* - ***Prenatal melatonin extends gestation and decreases fetal growth in high-altitude sheep***
E. Herrera - Pathophysiology Program, Faculty of Medicine; Universidad de Chile, Santiago, Chile

1.00 pm *Closing Remarks and Awards Presentation*
*Awards Committee*
*President: Tim Moss*
*Members: Tullia Todros, Dino Giussani*

1.30 pm *Lunch*





# Annual Geoffrey Dawes lecture

**Emerging Problems in Plant Pathology: Alien Species and Human Pathogens**

**Maria Lodovica Gullino**
AGROINNOVA and DISAFA, University of Turin, Via Leonardo da Vinci, 44, 10095, Grugliasco, Italy

The epidemic outbreak of new plant diseases can have negative consequences on agriculture, the environment, international trade and, in the worst cases, even ripercussions of social nature. There are various hypotheses as to the origin of these diseases: imports from other countries, epidemic outbreaks of diseases already endemically present, expansion of the areas in which the pathogens spread, as a consequence of climate change, adaptation of pathogens to new hosts, changes in cultural and management practices.
Strictly speaking, those diseases whose incidence has risen over the last 15-20 years can be defined as emerging diseases. Emerging diseases can appear for the first time or can spread in new areas for the first time. In this review, the term is used broadly to comprise the following categories: new diseases, re-emerging diseases, chronic/spreading diseases and threatening diseases. By “new diseases” we mean the diseases reported for the first time on a host in a new geographical area in the last 5 years; “re-emerging” is a term used to define a disease which is already known in a certain geographical area, but which suddenly becomes relevant for various reasons such as the appearance of resistance to fungicides, the substitution or introduction of a crop, or variations in the populations of endemic pathogens. Finally, in the “chronic/spreading disease” category we define the diseases which are endemically present in a certain area, but occasionally cause an epidemic outbreak. The “threatening diseases” are those that have not yet been reported in a geographical area or have a limited distribution. Among these are the diseases caused by pathogens in quarantine.
The phytopathological situations of horticulturakl crops and the problems caused by alien species as well as by human pathogens able to contaminate and, in some cases, enter vegetables will be critically discussed.
The relationship between the presence of alien species and contamination by human pathogens with the current limitations in the use of chemicals, the globalization of the markets and/or of climate change will be critically discussed.

# ABSTRACTS

# ORAL COMMUNICATIONS

## Oral Session I - Fetal & Neonatal Cardiovascular (I)

***Chairs***
***Dino Giussani*** *(University of Cambridge - UK)*
***Luc Zimmermann*** *(Maastricht University - Netherlands)*





O1

## Adenosine A2 receptors and nitric oxide-dependent signaling pathway may be involved in fetal endothelial cells proliferation during gestational diabetes

**Escudero Carlos [1], Acurio Jesenia [1], Troncoso Felipe [1], Escudero Andrea [2], Bertoglia Patricio [3]**

[1] Vascular Physiology Laboratory, Group of Investigation in Tumor Angiogenesis, Group of Research and Innovation in Vascular Health, Department of Basic Sciences, Universidad del Bio Bio, Chillan, Chile
[2] Facultad de Ingeniria, Carrera de Ingeniería Agroindustrial, Universidad Nacional de Chimborazo, Riobamba, Ecuador
[3] Obstetric and Gynecology Department, Hospital Clinico Herminda Martin and Universidad Católica de la Santísima Concepción, Chillan, Chile

**Aim:** Characterize whether $A_{2A}$ and/or $A_{2B}$ adenosine receptors (AR) via nitric oxide (NO) signaling pathway are involved in proliferation of fetal endothelium isolated from pregnancies with gestational diabetes (GD).

**Methods:** Analysis were performed in human umbilical vein endothelial cells (HUVEC) isolated from normal pregnancies (n=18) and GD (n=15). Cell proliferation was assayed by cell counting and MTS in presence (24 h) or absence of non-selective AR agonist (NECA 10μM), $A_{2A}$AR selective agonist (CGS-21680, 30nM), and/or the antagonists ZM-241385 (10 nM) and MRS-1754 (10 nM) for $A_{2A}$AR and $A_{2B}$AR, respectively. NECA was also combined with the non-selective nitric oxide synthase (NOS) inhibitor L-NAME, (100 μM) or the NO donor, SNAP (10 pM). Finally, cells were transfected with either shRNA-$A_{2A}$AR and/or shRNA-$A_{2B}$AR or respective control (i.e., shRNA-A). Twenty-four hours post-transfection, cells were used for analysis of cell proliferation in presence or absence of NECA or SNAP during additional 24 hours. All experiments were performed in presence of adenosine deaminase (1 UI/ml).

**Results:** GD was associated with high (~2 fold) $A_{2A}$AR and $A_{2B}$AR protein level. CGS-21680 and NECA increase (~1.3 and 1.5-fold) cell proliferation in both GD and normal pregnancies. NECA-stimulatory effect observed in GD or normal cells was partially blocked by ZM-241385 or MRS-1754 by separates, whereas additive inhibition was observed when both antagonists were co-incubated. Furthermore, knocking down of either $A_{2A}$AR or $A_{2B}$AR by separate did not block the stimulatory effect of NECA on cell proliferation, being ~1.3 fold higher than control in both normal and diabetic cells. Only, combination of shRNA-$A_{2A}$AR and shRNA-$A_{2B}$AR blocked the stimulatory effect of NECA in both normal and GD. Nevertheless, L-NAME co-incubation blocked the NECA-stimulatory effect in both groups of pregnancies. SNAP alone slightly increased cell proliferation but not reach statistical significance in either normal pregnancy or GD. No additive effect was observed in cell proliferation when CGS-21680 or NECA were co-incubated with SNAP. In cell co-incubated with SNAP and NECA, cells lacking of $A_{2A}$AR or $A_{2B}$AR receptor exhibited similar response that NECA alone in both groups of pregnancies. Whereas, cell lacking of both $A_{2A}$AR and $A_{2B}$AR did not response to SNAP.

**Conclusions:** Elevated expression of both $A_{2A}$AR and $A_{2B}$AR may generate cell proliferation in gestational diabetes. Participation of NO during this process needs further studies. Supported by FONDECYT 1140586.

O2

## Chronic hypoxia in the growth-restricted fetus is associated with FHR overshoot during brief repeated umbilical cord occlusions in near-term fetal sheep

Wassink Guido [1], Lear Christopher [1], Bennet Laura [1], Gunn Alistair Jan [1]

[1] Department of Physiology, Faculty of Medical and Health Sciences, The University of Auckland

**Introduction:** Spontaneous antenatal hypoxia particularly in growth-restricted fetuses is commonly associated with increased risk of still birth, metabolic acidosis during labour, and abnormal long-term neurodevelopment. The appearance of fetal heart rate overshoot has been proposed as a prognostic biomarker of developing metabolic acidosis during variable decelarations such as labour-like asphyxia.

**Methods:** Chronically instrumented fetal sheep (125 ± 3 days) were exposed to 1-minute umbilical cord occlusions every 5 minutes (normoxic group; $PaO_2$ > 17mmHg, n = 9, hypoxic group; $PaO_2$ < 17mmHg, n = 9) repeated for 4 hours or until arterial blood pressure fell below 20mm Hg during two succesive occlusions. The hypoxic group was smaller (3258g ± 599 *vs.* 4043g ± 373, p<0.01), and contained more twins (7 *vs.* 4). Overshoot was defined as an acceleration of fetal heart rate (>15 bpm over baseline) within 60 seconds after release of each occlusion for two subsequent occlusions.

**Results:** The normoxic group tolerated 4 hours of brief repeated occlusions without hypotension or clinically significant metabolic acidosis, and overshoot never occurred.
In contrast, the hypoxic fetuses became progressively acidotic and hypotensive and occlusions were terminated early at 201 ± 10.4 minutes in 5 of 9 fetuses. Overshoot was observed in all fetuses (46.03 ± 5.91 bpm, p<0.001), and was present from the first occlusion onwards in 5 of 9 fetuses; the remaining fetuses developed overshoot from occlusion 2, 3, 5 and 23 onwards. Baseline pH and glucose was lower (p<0.01), while PaCO2 and lactate were higher (p<0.05) compared with the normoxic group, but severity of acidosis did not change the onset or magnitude of overshoot.

**Conclusion:** Chronic hypoxia in growth-restricted fetuses resulted in progressively severe compromise during mild labour-like asphyxia, and was associated with early onset of fetal heart rate overshoot. These results suggest that overshoot indicates reduced cardiac tolerance to labour-like insults, but does not identify compromise.





O3

## Altered cardiopulmonary and cerebral hemodynamic responsiveness to early inhaled nitric oxide in growth restricted preterm lambs

Polglase Graeme [1,2], Miller Suzanne [1,2], Gill Andrew [3], Kluckow Martin [4], Jenkin Graham [1,2], Coia Elise [1,2], Allison Beth [1,2], Malhotra Atul [1,2], Sehgal Arvind [1,2] Hooper Stuart [1,2]

[1] The Ritchie Centre, Monash Institute of Medical Research – Prince Henrys Institute, Melbourne, Australia
[2] Department of Obstetrics and Gynaecology, Monash University, Melbourne, Australia
[3] Centre for Neonatal Research and Education, The University of Western Australia, Perth, Australia
[4] Department of Neonatalogy, Royal North Shore Hospital and University of Sydney, Sydney, Australia.

**Introduction:** Inhaled nitric oxide (iNO) is a potent vasodilator used clinically to reduce pulmonary hypertension in preterm infants. However, the efficacy of iNO in growth restricted preterm infants is not known.

**Materials and methods:** Ewes bearing twins underwent ligation of a single umbilical artery at 105 days gestation (term ~ 148 d) to induce growth restriction (FGR); the second twin was used as an internal control (n=8 each). At ~123 d, lambs catheters and flow-probes were implanted for measurement of pulmonary (PBF) and cerebral arterial blood flows (CBF) and arterial pressure ($P_{CA}$). Lambs were delivered, and ventilated (Drager Babylog 8000+) for 2 h. At 60 min, inhaled nitric oxide (iNO; 20 ppm) was administered for 30 min by inhalation. Ventilation, oxygenation and hemodynamic responses were recorded continuously. Cardiovascular responses to iNO were assessed using Doppler Echocardiography.

**Results and discussion:** FGR resulted in a 26 % reduction in body weight (p<0.05). Ventilation, respiratory parameters and cardiopulmonary and cerebral hemodynamics did not change in the first 60 min; no lambs presented with pulmonary hypertension. In FGR lambs within 10 minutes of administration of iNO, PBF increased by 35 %, left ventricular output by 222 %, systemic arterial pressure by 24 % and CBF by 25.6%. However, iNO decreased LVO by 26 %, arterial pressure by 19 % change and reduced CBF by 44.3 % in controls. Ductal steal, evidenced by reversed diastolic CBF, was observed in all control lambs during iNO administration.

**Conclusion:** FGR preterm lambs have an increased pulmonary vascular responsiveness to iNO, resulting in improved cardiovascular, systemic and cerebral hemodynamics. Conversely, iNO administration to control preterm lambs had deleterious consequences, particularly to CBF. Understanding the hemodynamic status of the preterm neonate prior to iNO administration is critical to preventing adverse consequences.

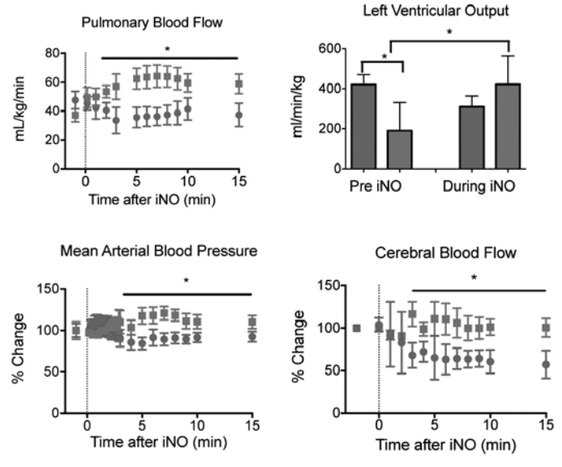



Figure 1: Influence of iNO on haemodynamics in preterm lambs.
The initiation of inhaled nitric oxide (iNO; dotted line) results in a rapid increase in pulmonary blood flow, left ventricular output and arterial pressure in FGR lambs (blue squares). However, iNO administration had the opposite effect in AG preterm lambs (red circles) and also halved cerebral blood flow within 5 minutes of administration.

O4

## Foetal and umbilical vascular reactivity in a model of IUGR through gradual uterine artery occlusion in guinea pigs

**Schneider Daniela [1], Alegría René [2], Farías Marelo [1], Herrera Emilio A [2], Casanello Paola [1], Krause Bernardo J [1]**

[1] Division of Obstetrics and Gynecoloy, School of Medicine, Pontificia Universidad Catolica de Chile, Santiago, Chile

[2] Laboratorio de Función y Reactividad Vascular, ICBM, Universidad de Chile, Santiago, Chile

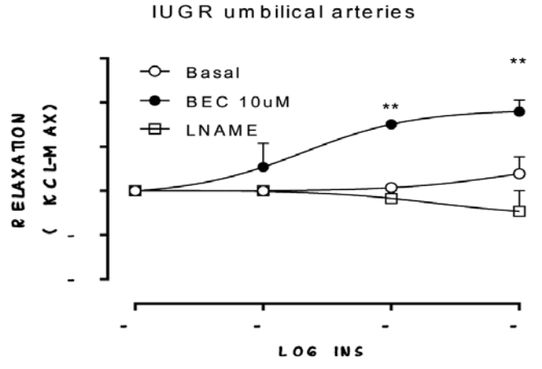
**Iugr:** relates with altered placental vascular reactivity and increased cardiovascular risk in the neonate, however whether vascular changes observed in umbilical arteries reflects the alterations present in systemic arteries in the IUGR foetus in unknown.

**Objectives:** In pregnant guinea pigs we aimed to determine the effect of gradual occlusion of uterine artery on foetal growth, and the NO-dependent relaxation in umbilical and systemic (aorta) arteries in IUGR foetuses.

**Methods:** IUGR was induced by implanting ameroid constrictors in uterine arteries at 40 days of gestation. Foetal growth and umbilical blood velocity was followed by Doppler Sonography. At day 63 foetuses were extracted, weighted and dissected. NO-dependent vasoactive responses to SNP (NO donor), and insulin or acetylcholine in presence or absence of L-NAME (NOS inhibitor) and BEC (arginase inhibitor) were studied by wire myography. The effects of pro- (SIN-1, $ONOO^-$ donor; MS, GPx inhibitor; DDC, SOD inhibitor) and antioxidant (NAC, N-acetylcysteine) agents were determined.

**Results:** Uterine artery occlusion reduced foetal (~50%), placental (~35%) and liver (~60%) weight, and increases umbilical artery resistance during gestation compared to control. IUGR umbilical arteries showed a lower insulin-induced relaxation (7.2 ± 10.2%KCl vs. 41.3 ± 7.0%KCl), and increased sensitivity to SNP (7.17 ± 0.29 vs. 6.08 ± 0.07). IUGR aortas showed higher response (57.2 ± 0.7%KCl vs. 22.5 ± 0.9%KCl) and decreased sensitivity (5.86 ± 0.05 vs. 6.89 ± 0.13) to acetylcholine, and higher relaxation to SNP (103.5 ± 5.3%KCl vs. 70.6 ± 9.5%KCl) compared to controls. NAC or BEC increased eNOS-dependent relaxation, whilst SIN-1, MS and DDC reduced this effect in umbilical arteries and aortas.

**Conclusions:** Guinea pig IUGR foetuses present altered NO-mediated vascular reactivity in umbilical arteries and aorta, characterized by a higher NO sensitivity and decreased sensitivity to eNOS-dependent agonist. Notably, changes in umbilical artery reactivity in IUGR guinea pigs are comparable to those observed in human IUGR umbilico-placental vessels.




*Funded by* FONDECYT 1130801





O5

## The mitochondrial targeted antioxidant MitoQ and Sildenafil rescue endothelial dysfunction in the hypoxic chick embryo

**Skeffington K.L. [1], Itani N. [1], Beck C. [1], Logan A. [2], Murphy M.P. [2], Giussani D.A. [1]**

[1] Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, United Kingdom

[1] Mitochodrial Biology Unit, Addenbrookes Hospital, Cambridge, United Kingdom

**Introduction:** Chronic fetal hypoxia programmes endothelial dysfunction at adulthood through the generation of oxidative stress *in utero* and maternal treatment with vitamin C in hypoxic pregnancy prevents the programing effects (1). However, only high doses of vitamin C incompatible with human therapy were effective. Therefore, there is interest in alternative antioxidant strategies to treat fetal origins of vascular dysfunction during hypoxic pregnancy in humans. We compared the effects on peripheral vascular reactivity of treatment of chick embryos undergoing normoxic or hypoxic incubation with MitoQ or Sildenafil .

**Materials and methods:** Fertilised eggs were incubated under normoxia (21%) or hypoxia (14±0.5%) from day 0 with or without MitoQ or Sildenafil treatment from days 13 to 18 of incubation. Sildenafil (4 $mg.kg^{-1}$), MitoQ (0.2 $mg.kg^{-1}$) or vehicle (water) was injected daily into the air cell through a 1 mm hole in the shell. At day 19, the embryo was killed by spinal transection. Following biometry, femoral arterial dilator reactivity to acetylcholine (ACh; $10^{-9.5}$-$10^{-4}$ $mol.L^{-1}$) was investigated ± L-NAME ($10^{-5}$ $mol.L^{-1}$) and ± indomethacin ($10^{-6}$ $mol.L^{-1}$) in a wire myograph .

**Results and discussion:** Both MitoQ and Sildenafil improved endothelial function but did not reverse growth restriction in hypoxic embryos (Fig. 1). Sildenafil improved femoral relaxation via NO-dependent mechanisms (area under the curve (AUC) for NO availability 54.4 ± 8.1 (hypoxic vehicle) *vs.* 79.3 ± 6.3 (hypoxic Sildenafil, $P<0.05$). Hypoxic MitoQ treated embryos showed greater prostanoid-dependent relaxation compared to hypoxic vehicle embryos (AUC 55.9 ± 10.0 *vs*. 28.6 ± 4.7, $P<0.05$). MitoQ treatment also increased NO at high doses of Ach (AUC [Ach] $10^{-4.5}$-$10^{-4}$ 5.5 ± 1.8 *vs.*0.1 ± 1.6, $P<0.05$).

**Conclusion:** MitoQ or Sildenafil rescued endothelial dysfunction in hypoxic development. The work identifies potential antioxidant candidates for translation to human therapy against fetal origins of cardiovascular disease in high risk pregnancy.

*Supported by the British Heart Foundation*

1. Giussani et al. *PLoS ONE* 7(2):e31017, 2012

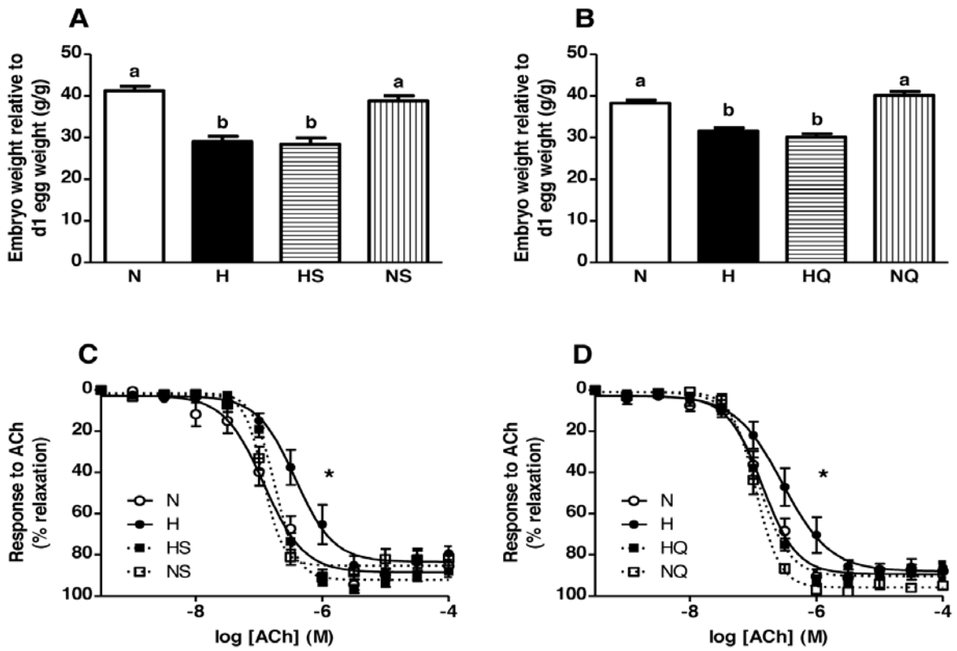



Figure 1. Means ± S.E.M. for fetal weight at the end of the incubation period as a percentage of the initial egg mass (A and B) and the concentration-response curve to acetylcholine (ACh) expressed as percentage relaxation (C and D). Treatment groups are N, normoxic incubation, H, hypoxic incubation, HS, hypoxic incubation with Sidenafil, NS, normoxic incubation with Sildenafil, HQ, hypoxic incubation with MitoQ and NQ, normoxic incubation with MitoQ . A; N, n=10, H, n=10, HS, n=14 and NS, n=11. B; n=30 in all groups. C; n=8, n=10, n=10 and n=10 respectively. D; n=8 in all groups. *P<0.05, pD2 vs. N (ANOVA with Tukey Test or SNK tests).





O6

## The development of primary hemostasis and platelet function during mouse fetal life in vivo

**Margraf Andreas [1], Nussbaum Claudia [1], Rohwedder Ina [1], Dietzel Steffen [1], Sperandio Markus [1]**

[1] Walter Brendel Center of Experimental Medicine, Ludwig Maximilians University, Munich, Germany

**Introduction:** Platelets are known to play a substantial role during primary hemostasis. While this process is well investigated in adult mammalian organisms, it remains unclear how platelet function and primary hemostasis develops during fetal ontogeny.

**Methods:** Using a newly developed intravital microscopy thrombosis model, thrombus formation was observed in fetal yolk-sac vessels in fetuses between ages E13.5 and E17.5 (out of 21 days of gestation). Platelet adherence (onset), thrombus stability (reflow) and firm vessel occlusion were analyzed. Multiphoton-imaging was applied to study anatomical properties of yolk-sac vessels. All surgical procedures were performed under anesthesia (ketamine/xylazine). FACS analysis was used for measurement of platelet surface expression patterns and platelet counts. P-selectin granule content was determined by western blotting.

**Results:** Thrombus onset and firm vessel occlusion both appeared significantly less frequent in youngest fetuses compared to older ones (p<0,05). Additionally, reflow after primary thrombus formation could be noted more frequent in E13.5 fetuses. Time until platelet adhesion and thrombus formation was prolonged in youngest fetuses compared to older ones. FACS-analysis revealed significantly lower platelet counts in fetal blood samples compared to adult levels. Furthermore, impaired platelet activation upon stimulation with thrombin could be noted in fetuses compared to adult mice.

**Conclusion:** We identified decreased thrombus formation and thrombus stability during early fetal development in vivo with an increase in thrombogenic responses in more mature fetuses. These results may not only improve our understanding of primary hemostasis during fetal life, but also stimulate the development of new therapeutic strategies in neonatal diseases including intraventricular hemorrhage or persistent ductus arteriosus.

O7

## Vasoactive effects of serotonin in the ductus arteriosus of the chicken embryo

**van Zogchel Lieke, Villamor Eduardo**

Department of Pediatrics, School for Oncology and Developmental Biology (GROW), Maastricht University Medical Center (MUMC+), Maastricht, Netherlands

**Introduction:** Decreased platelet number and/or function have been related to patent ductus arteriosus (DA). Activated platelets release vasoactive products, including serotonin (5-HT), that might be relevant for DA homeostasis DA. The chicken embryo has emerged as a suitable model for the study of DA vascular biology. In the present study, we investigated the possible vasoactive role of 5-HT in the chicken DA.

**Methods:** Rings of the DA of 15- to 20-d-old chicken embryos (total incubation time 21-d) were studied in a wire myograph. The response to 5-HT was investigated under different $O_2$ tensions (3, 7, and 74 kPa). The responses to the $5\text{-HT}_{1B/D}$ receptor agonist sumatriptan, the $5\text{-HT}_{2A/B/C}$ receptor agonist DOI and the selective serotonin reuptake inhibitors fluoxetine and sertraline were also investigated.

**Results:** 5-HT (10 nM-0.3 mM) contracted the pulmonary side of the DA (PulmDA) in a concentration-dependent manner. By contrast, 5-HT induced negligible contractions in the vessels that surround the PulmDA (i.e., the pre- and post-ductal pulmonary arteries, and the aortic side of the DA). 5-HT-induced contraction increased with development (15-d>17-d>19-d=20-d). $O_2$ tension did not affect 5-HT-induced contraction but elimination of extracellular calcium completely abolished it. Sumatriptan and DOI also contracted the PulmDA in a concentration-dependent manner. High concentrations (>0.1mM) of Fluoxetine and sertraline evoked contraction in PulmDA rings pre-contracted with $O_2$. By contrast, fluoxetine relaxed PulmDA rings under basal tone or pre-contracted with KCl or 5-HT.

**Conclusions:** Our data indicate that 5-HT receptors are functionally present in the chicken DA and suggest that platelet-derived 5-HT may play a pivotal role in the postnatal closure of the DA.





O8

## Doppler blood flow variables in the human fetal middle cerebral (MCA), right pulmonary (RPA) and umbilical arteries (UA): evidence for fluctuating arterial Po2

**Stebbings Eleanor, Lim Ken, Oberlander Tim F, Brain Ursula, Rurak Dan**

Child & Family Research Instiute, University of British Columbia, Vancouver, Canada

In the fetal lamb, there are continuous fluctuations in arterial $Po_2$ and other blood gas variables. The fluctuations in $Po_2$, which are largely transient decreases, are associated with episodic fetal somatic activity, uterine contractions and changes in maternal position from lying to standing. At times, the decreases in $Po_2$ are severe, and, when changes of similar magnitude are achieved in experimental fetal hypoxemia, they elicit changes in cardiovascular function, in particular decreased MCA vascular resistance and pulmonary vasoconstriction. In the human fetus, there are episodic fetal activity, uterine contractions and frequent changes in maternal position. However, whether there are decreases in fetal vascular $Po_2$ associated with these events cannot currently be determined directly, because of technical and ethical constraints. We reasoned that if such changes in fetal $Po_2$ do indeed occur, they should lead to transient cerebral vasodilation and pulmonary vasoconstriction, which could be determined with longitudinal Doppler ultrasound assessments in association with monitoring of fetal activity and uterine contractions. Moreover, in cross-sectional Doppler ultrasound data, fluctuation fetal $Po_2$ might be manifested by an inverse relationship between resistance indices (e.g. pulsatility index, PI) in the cerebral and pulmonary circulations, since some of the estimates would likely have been obtained shortly after fetal activity bouts or uterine contractions and others during fetal quiescence. In the current study, we examined these Doppler ultrasound variables obtained from a project examining the fetal effects of SSRI antidepressant exposure. Only data from the control, health pregnancy group (n= 47) were analyzed. These women had singleton pregnancies, with a mean gestational age of 40.0±0.2 weeks and a mean birth weight of 3534±64 g. The Doppler ultrasound estimates were obtained at 36 weeks gestation twice during the day (am and pm), and comprised measures of PI, vessel diameter, and volume flow in the MCA and RPA and PI only in the UA. The am and pm values were combined. PI values in MCA, RPA and UA averaged 1.72±0.04, 2.92±0.08 and 0.93±0.02, and these were significantly different from each other ($p<0.001$). In both the MCA and RPA, volume flow was inversely related to PI, with the best fit of the data being with 1/PI (MCA flow = (449.6/PI) -102.1, $r^2 = 0.241$, $p < 0.001$; RPA flow = (539.8/PI) + 18.3, $r^2 =.209$, $p < 0.001$). There was a significant inverse relationship between MCA PI and RPA PI (RPA PI = -(.502 x MCA) + 3.78, $r^2 =0.053$, $p = 0.039$). In contrast, there was a significant positive relationship between MCA PI and UA PI (UA PI = (0.128 x MCA PI) +0.70, $r^2 = 0.069$, $p = 0.007$). We conclude that the inverse relationship between MCA and RPA PI values may be due to periodic decreases in fetal vascular Po2, and that the positive relationship between MCA and UA PI values may reflect an increase in umbilical blood flow that often occurs during fetal activity bouts and which may in part be due to umbilical vasodilation. Further validation of these hypotheses could be obtained by longitudinal measurement of these Doppler ultrasound variables.

# ORAL COMMUNICATIONS

Oral Session II - Developmental Origins of Health and Disease

***Chairs***
***Laura Bennett*** *(University of Sidney, Australia)*
***Donald Peebles*** *(University College London, UK)*





O9

## Growth and endothelial function in the first 2 years of life: a longitudinal study

**Touwslager RNH [1], Houben AJHM [1], Tan FES [1], Gielen M [1], Zeegers MP [1], Stehouwer CDA [1], Gerver WJM [1], Westerterp KR [1], Wouters L [1], Blanco CE [1], Zimmermann LJI [1], Mulder ALM [1]**

[1] MUMC, Maastricht, Maastricht, Netherlands

**Background:** Accelerated infant growth is related to later cardiovascular disease risk. We hypothesized that the harmful association between infant growth and endothelial function at 6 months we identified earlier would persist to the age of 24 months and, second, that accelerated growth would lead to an increased fat percentage and that this would in turn impact negatively on endothelial function.

**Methods:** In a prospective study 104 healthy term newborns underwent anthropometry and measurements of vascular vasodilatation at 0, 6, 12 and 24 months. We recorded maximum vasodilatation in response to acetylcholine (endothelium-dependent) and nitroprusside (endothelium-independent) by use of laser-Doppler vascular perfusion monitoring of the forearm skin vasculature. Additional anthropometry at 1 and 3 months was collected from child welfare centers. The data were analyzed by multilevel linear regression.

**Results:** Weight gain from 0-1 month was associated inversely with maximum perfusion in response to acetylcholine (b=-8.28 PU per Δ z-score, *P*=0.03). Weight gain from 0-1 month was related positively to maximum perfusion in response to nitroprusside (b=10.12 PU per Δ z-score, *P*=0.04), as was birth weight (b=8.02 PU per z-score, *P*=0.02). Fat percentage did not have a significant effect in any of the perfusion models and was not related to maximum perfusion at 2 years.

**Conclusions:** Infant weight gain from 0-1 month is inversely related to endothelial function in healthy term infants, at least up till the age of 2 years. This relationship was not likely to be explained by an increased fat percentage.

O10

## Cardiac structural and functional changes in fetal and adult offspring of hypoxic pregnancy can be prevented with maternal antioxidant treatment

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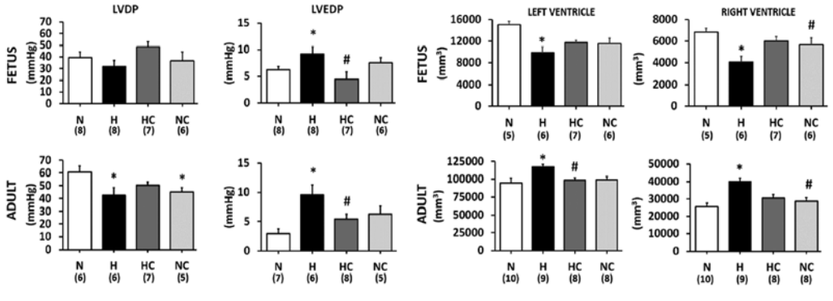
**Introduction:** Stressors during early life can programme heart disease. It is understood that they do so by altering adaptive responses to later challenges, such as a sedentary lifestyle, rather than by initiating pathological changes *per se* during fetal development. Whether suboptimal environments trigger a fetal origin of cardiac dysfunction has been difficult to prove in mammals, largely due to the complexity of studying fetal cardiac function in rodents. We have created isobaric chambers able to maintain pregnant sheep under hypoxic conditions for the duration of pregnancy. We have used this model to investigate the effects of chronic fetal hypoxia on cardiac structure and function in the offspring both during the fetal and adult periods and to test whether maternal antioxidant treatment is beneficial.

**Methods**: Ewes (n=5-8/group) carrying a singleton fetus were instrumented with catheters at 100 days of gestation (term~145 days). Half of the pregnancies were exposed to chronic hypoxia (10%, maternal $PO_2$:107±2 to 47±1 mmHg) from 105 days to term ± maternal vitamin C (200 mg/kg i.v. daily). Hearts of fetal and adult offspring were isolated at 138 days or at 9 months, respectively. Cardiac function was investigated using Langendorff preparations. Cardiac structure was assessed in fixed tissue using stereological techniques.

**Results:** In fetal sheep, hypoxic pregnancy triggered diastolic dysfunction (LVEDP) and a decrease in biventricular volume. By adulthood, diastolic dysfunction was accompanied by systolic dysfunction (LVDP) and ventricular volumes were increased, indicative of hypertrophy. Maternal vitamin C treatment in hypoxic pregnancy restored cardiac structural and functional changes in fetal and adult offspring.

**Conclusions:** Our data show that cardiac dysfunction is already evident by the end of hypoxic pregnancy, but amplifies with age. Maternal antioxidant treatment prevented the deleterious effects, thereby providing insight into mechanism and intervention in human high risk pregnancy.

*Supported by The British Heart Foundation*




Values are mean ± S.E.M. Normoxic (N); hypoxic (H); hypoxic + vitamin C (HC) and normoxic C (NC) A. Left ventricular developed pressure (LVDP) and left ventricular end diastolic pressure (LVEDP). B. Left and right ventricular volumes. Two-way ANOVA with Tukey test. * vs. N, # vs. H.





O11

## Metabolic and morphological changes during obesity with and without pregnancy

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**Background:** Although maternal fat stores increase in all pregnant women, irrespective of pre-pregnancy weight, the storage capacity of subcutaneous adipose tissue (SAT) is impaired, and fat predominantly accumulates in visceral adipose tissue (VAT). VAT is an important risk factor for metabolic imbalance in human subjects, also during pregnancy. Adipocyte dysfunctions are the primary defects in obesity and may link obesity to disorders like increased risk of insulin resistance, type 2 diabetes, fatty liver disease, hypertension, dyslipidemia, atherosclerosis and cancer. We propose to deepen our knowledge of the VAT morphology (cell size distribution and inflammatory cells presence) and serum biochemical parameters in obese and control women with and without pregnancy.

**Methods:** We evaluated 10 obese and 10 controls with and without pregnancy women (mean body mass index [BMI] >30 kg/m$^2$ and <25 kg/m$^2$, respectively). We collected VAT and serum samples from all women. Conventional biochemical parameters were measured by routine laboratory procedures, and leptin and adiponectin by Luminex xMAP technology. Five-micron sections were prepared from all paraffin-embedded VAT blocks. Slides were then stained with hematoxylin & eosin. Macrophagic infiltration were evaluated by CD68 immunohistochemical analysis.

**Results:** Glucose and lipid metabolisms, liver markers as well as the leptin/adiponectin ratio were significantly lower in obese *vs* control women with and without pregnancy ($p<0.05$). The number of VAT adipocytes was greater and their size smaller in obese *vs* control women with and without pregnancy ($p<0.05$). Furthermore, CD68 score, a VAT inflammation marker, resulted significantly reduced in control *vs* obese women with and without pregnancy ($p<0.05$). Moreover, the morphological characteristics and CD68 score of VAT adipocytes did not differ between obese women with and without pregnancy (n.s.).

**Conclusions:** The morphological characteristics and the inflammation status in VAT resulted to be essentially influenced by the pre-existing obesity and not by the pregnancy status.

O12

## Sensitivity analysis for validation of a model to simulate fetal heart rate decelerations during labor

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**Introduction:** Insight in feto-maternal oxygenation is important to determine fetal condition during labor. Fetal heart rate (FHR) decelerations may be a sign of impaired oxygenation. Van der Hout et al developed a computerized model for simulation of the FHR pattern during labor.[1,2] Model validation is needed to enhance the models accuracy in the prediction of FHR patterns. This study focusses on the identification of variables that influence the uncertainty in FHR deceleration depth as predicted by the model.

**Methods:** The model consists of several elements, such as the fetal and maternal cardiovascular system, oxygen distribution and baro and chemoreceptor responses. We performed a sensitivity analysis to identify the individual contribution of eleven selected input variables to FHR (see table 1). Each of these variables is varied with up to 20% baseline deviation in a randomized order, by using the Morris screening method.[3] Baseline values are obtained from fetal, neonatal, adult and animal studies, as well as expert opinion and best guess.[4,5] As a result we obtained a ranking of input variables according to their effect on FHR deceleration depth.

**Results:** Table 1 shows the ranking of input variables that contribute most to the uncertainty in the prediction of FHR deceleration depth by the simulation model.

**Discussion:** Sensitivity analysis is an important tool to estimate to what extent input variables influence output variables in a simulation model. For our model, designed to simulate FHR pattern, all variables included in the screening are important contributors to the uncertainty in the prediction of FHR deceleration depth. To optimize the models accuracy, it is important to precisely define these specific input variables. Mainly the accurate estimation of fetal hemoglobin concentration is important, as this will mostly effect the uncertainty in the prediction of FHR deceleration depth.

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Table 1 Feto-placental input values ranked according to their effect in the uncertainty of the fetal heart rate deceleration depth. Parameters are ranked from most to least influential.

| Morris ranking | Parameter |
|---|---|
| 1 | Hemoglobin concentration |
| 2 | Oxygen concentration threshold during hypoxia |
| 3 | Fetal cardiac muscle volume |
| 4 | Parasympathetic gain |
| 5 | Placental oxygen diffusion capacity |
| 6 | Umbilical cord vascular resistance |
| 7 | Sympathetic gain |
| 8 | Cardiac contractility |
| 9 | Peripheral vascular resistance |
| 10 | Cerebral vascular resistance |
| 11 | Oxygen metabolism |

O13

## Intergenerational programming of heart disease and heritability of cardioprotection via the maternal mitochondria

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**Introduction:** The mechanisms via which parents transfer information about the environment they experience onto their offspring remain under discussion. We report in a rat model of intergenerational programming by developmental hypoxia the transmission of cardiac dysfunction via the paternal line. In contrast, adult females which experienced hypoxia *in utero* transmit onto their offspring a protection against this cardiac deficit. We show that the mechanism of maternal transmission of this cardioprotective trait is via the mitochondria.

**Methods:** Pregnant rats (n=24, F0) underwent normoxic (N) or hypoxic (H: 14% $O_2$) pregnancy from days 6-20 of gestation. At 12 weeks, F1 offspring were mated with partners from outside the colony to produce an F2 generation which did not experience hypoxia from both parental lineages. In F1 and F2 adult males, we determined cardiac recovery from ischaemia/reperfusion (I/R, Langendorff), cardiac mitochondrial $O_2$ consumption (permeabilised muscle fibre respirometry) and cardiac expression of protein kinase C epsilon (PKCe, Western blot).

**Results:** F1 and F2 paternal lineage offspring of hypoxic pregnancy showed impaired cardiac recovery from I/R (Fig. 1a). In contrast, F2 maternal lineage offspring of hypoxic pregnancy showed normal recovery to I/R. Cardiac protection to I/R in F2 maternal lineage offspring of hypoxic pregnancy was associated with a decrease in mitochondrial complex I $O_2$ consumption (Fig. 1b) and in a reduced fall in cardiac PKCe expression after ischaemia (Fig. 1c).

**Discussion and Conclusions:** A decrease in complex I $O_2$ consumption is an established mitochondrial antioxidant strategy to limit free radical generation in hypoxic tissue (1). The mechanisms of cardioprotection by PKCe are established to be mitochondrial (2). Therefore, the data show the intergenerational programming of heart disease via the paternal line and the heritability of cardioprotection via the maternal mitochondria. This may be a mechanism driving environmental adaptation from generation to generation.





*Support: British Heart Foundation*

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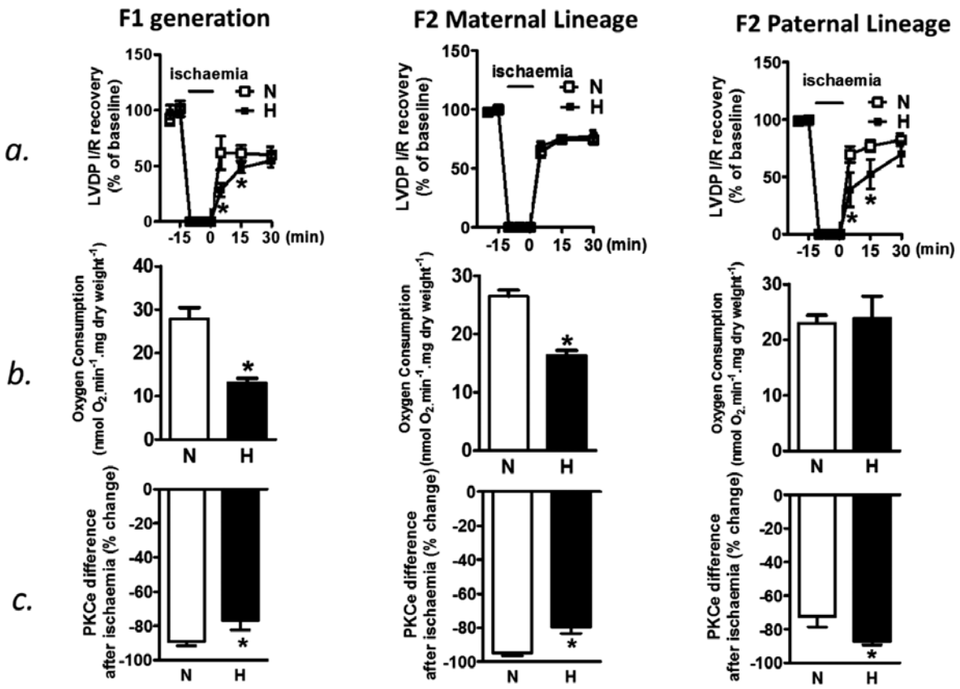


Figure 1. Recovery of left ventricular developed pressure (LVDP) after ischaemia (a), cardiac mitochondrial complex I State 3 oxygen consumption (b), and cardiac PKCε changes after ischaemia (c) in F1 and F2 male offspring of normoxic (N) or hypoxic (H) pregacy via paternal or maternal lineages. Values are mean ± S.E.M., n=6-10. *P<0.05, H vs. N, two way ANOVA with Tukey test.

O14

## Maternal iron status in early pregnancy and arterial stiffness in infants: an observational study

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**Background:** Iron deficiency during early pregnancy has been linked with increased offspring's cardiovascular risk in animal studies. Infant arterial stiffness, measured by pulse wave velocity (PWV) could offer a non-invasive and acceptable option for cardiovascular risk prediction later in life. No previous studies have measured PWV in the first weeks of life to examine its association with maternal micronutrient status in pregnancy. This study aimed to examine the association between maternal iron status during the first trimester of pregnancy, and infant brachio-femoral PWV (bfPWV) at 2-6 weeks of age.

**Methods:** Data from the Baby VIP (Baby's Vascular health and Iron in Pregnancy) study were used. This was a historical cohort which recruited 362 babies and their mothers after hospital delivery from the postnatal wards of Leeds Teaching Hospitals NHS Trust, UK. Serum ferritin (sF) concentrations were measured in maternal serum samples obtained in the first trimester of pregnancy. Maternal haemoglobin values were extracted from their antenatal records. Each baby's bfPWV was measured using the Vicorder device (Skidmore Medical) during a home visit by a research nurse at 2-6 weeks.

**Results:** Out of the pregnant women with information on sF in the first trimester (n=348), 23% (n=79) had iron depletion according to the WHO cut-off of 15 ug/l. The prevalence of anaemia at ≤ 20 weeks (<11 g/dl) and >20 weeks gestation (<10.5 g/dl) was 5% (16/329) and 14% (48/337) respectively. Mean infant bfPWV was 6.7 m/s (standard deviation=1.3, n=284). There was no evidence of association between infant bfPWV and maternal sF analysed as a continuous variable (adjusted change in PWV in m/s per 10 ug/l change in sF = 0.02, 95% CI -0.01, 0.10, P=0.3), nor with maternal iron depletion (<15 ug/l) ((adjusted change in PWV in m/s = -0.19, 95% CI -0.55, 0.18, P=0.3). Maternal anaemia at ≤ 20 weeks gestation was associated with a 1.00 m/s increase in infant PWV (adjusted 95% CI 0.14, 1.85, P=0.02).

**Conclusion:** This study demonstrates that arterial stiffness in infants can be feasibly assessed using non-invasive techniques of measuring PWV in population studies. Increased infant arterial stiffness in the first few weeks of life was associated with maternal anaemia in the first half of pregnancy, but not with iron depletion in the first trimester. Further investigation of this relationship using other measures of iron status such as serum transferrin receptor levels is required.





O15

## Induced prematurity in the guinea pig; a new model to study the life-course effects of preterm birth

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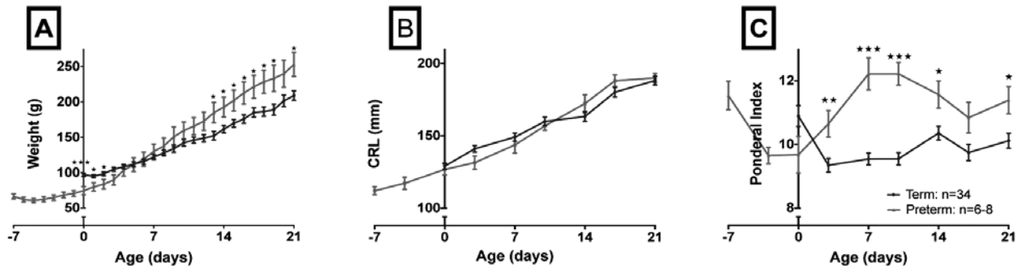
Preterm birth is associated with an increased risk of long-term cardio-metabolic dysfunction. A number of different animal models have been developed to investigate the mechanisms mediating this risk. However, their translation to the human situation is limited by differences in placental function, *in-utero* growth characteristics, relative maturity at term and preterm viability, necessity for operative delivery with orphan-rearing of offspring or the logistical constraints of long-term large-animal studies. Operative birth and early maternal care also influence long-term cardio-metabolic function.
Guinea-pigs may be a useful translational model of prematurity. Similar to humans, they have a haemochorial placenta, accrue fat during late-gestation, and short-term survival after preterm Caesarean is possible. They mature rapidly, enabling true life-course as well as intergenerational studies.

**Aim:** To develop a guinea-pig model of long-term survival following medically-induced prematurity.

**Methods:** Preterm parturition was pharmacologically induced at day 62 of a 69-day pregnancy. Pups were resuscitated and given subcutaneous fluids and syringe-fed milk until able to suckle. Until term equivalent age (TEA) pups and sows remained in a temperature- and humidity-controlled incubator. Term-born pups received standard laboratory care. Size was measured regularly until weaning at 21 days corrected postnatal age. Ponderal index (PI) was calculated (weight/length$^3$). Growth was assessed using ANOVA in same-sex groups; $p<0.05$ was significant.

**Results:** Preterm pups were fully suckle-fed by TEA and most survived to weaning (preterm 70% vs. term 94%). At TEA preterm-born pups were lighter, but by weaning, heavier with a greater PI than term-born pups (weight: 253±17g vs. 209±7g; PI: 11.4±0.4 vs.10.2±0.2; both $p<0.05$).




Growth to weaning of male preterm and term-born guinea pigs. A; weight. B; crown rump length. C; ponderal index. Data are mean ± SEM. *p<0.05, **p<0.01, ***p<0.001

**Discussion:** Long-term survival following induced prematurity in guinea-pigs is possible with intensive care analogous to that received by a moderate-preterm infant. Induced parturition removes the potentially confounding effects of operative delivery and orphan-rearing /cross-fostering. Ex-preterm pups are phenotypically different and represent a good translational model of human prematurity.





O16

## Risk of respiratory morbidity and mode of delivery in late preterm infants

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**Objective:** to assess the impact of mode of delivery on respiratory morbidity among late-preterm neonates.

**Methods:** Singleton pregnancies complicated by premature rupture of membranes (PROM) between 34+0 and 36+6 weeks were studied prospectively. Pregnancies with corticosteroid administration were excluded. Patients were divided into cesarean section (CS) and vaginal delivery groups, matched 1:3 for gestational age. The primary outcome was the rate of respiratory distress syndrome (RDS). Logistic regression was performed to assess the risk of RDS within groups.

**Results:** Between January 2005 and May 2014, 380 patients delivered between 34 and 36 weeks after PROM at St.Orsola-Malpighi Hospital, Bologna (Italy). In 95 cases elective caesarean section was performed for previous CS (n=48), breech presentation (n=29) or maternal medical indications (n=18). The overall RDS rate was 12,8%, while it was 23,16% and 9,12% in case of CS and vaginal delivery, respectively (p-value 0.0006). CS seems to be a risk factor for RDS (OR 3.79, p-value <0.0001), as does earlier gestational age at delivery (OR 0.32, p-value< 0.0001). Table 1 shows the median risks of RDS in the study population according to the logistic regression model.

**Conclusions:** After late preterm PROM, CS is associated with a higher risk of neonatal RDS. This is more evident with increasing gestational age, when respiratory morbidity is thought to be less frequent.

Table 1. median estimated risk of RDS

| | 34 weeks | 35 weeks | 36 weeks |
|---|---|---|---|
| CS (n=95) | 57.3% | 30.3% | 12.4% |
| Vaginal delivery (n=285) | 26.1% | 10.3% | 3.5% |

# ORAL COMMUNICATIONS

Oral Session III - Placenta & Miscellaneous

***Chairs***
***Claire Roberts*** *(University of Adelaide, Australia)*
***Chiara Mandò*** *(University of Milan, Italy)*





O17

## Elevated endothelial cell markers and tyrosine 1175 phosphorylation of vascular endothelial growth factor receptor type 2 in placentas from gestational diabetes

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**Introduction:** Since gestational diabetes has been associated with elevated placental angiogenesis, we aim to investigate whether placentas from diabetes exhibit elevated activation of vascular endothelial growth factor receptor type 2 (VEGFR2), characterized by tyrosine 951 and/or 1175 phosphorylation, compared to normal pregnancy.

**Materials and methods:** We obtained placental samples from normal pregnant women (n=12) and gestational diabetes (n=14), which were homogenized in order to obtain mRNA and protein extractions. Semi quantitative PCR was performed for estimating mRNA levels of VEGF, VEGFR type 1 (VEGFR1) and VEGFR2. Also, protein concentrations of CD31, CD34, VEGF, VEGFR1, VEGFR2 and Y951 phosphorylated VEGFR2 were estimated by western blot. Y1175 phosphorylated VEGFR2 was measured by ELISA.

**Results and discussion:** Non-significant differences were observed in the clinical parameters (Table) between both groups. Compared with placentas from normal pregnancies, those derived from gestational diabetes showed elevated protein levels of the endothelial cell markers, CD31 and CD34 (~1.5 and 2.1 fold, respectively). No significant changes were observed in the mRNA and protein levels of VEGF between normal and diabetic placentas. However a significant reduction in the mRNA levels of VEGFR1 (~41%) and VEGFR2 (~35%) without changes in protein abundance was observed in diabetes compared with normal pregnancy. Phosphorylation of Y1175 was elevated (~1.3 fold, p=0.05), whereas phosphorylation of Y951 (~10%, p=0.02) in the VEGFR2 was reduced in gestational diabetes compared with normal pregnancy.

**Conclusion:** The elevated protein level of proangiogenic markers observed in placentas from gestational diabetes is associated with increased phosphorylation of Y1175 of VEGFR2. A compensatory down regulation in the mRNA levels of VEGFR1 and VEGFR2, as well as Y951 phosphorylation of VEGFR2 may be also taken part in gestational diabetes.

Table. Clinical characteristics of included patients

| | Normal Pregnancy (n=12) | Gestational Diabetes (n=14) |
|---|---|---|
| **Maternal** | | |
| BMI before pregnancy (Kg/cm$^2$) | 26.1±1.3 | 30.6±1.8 |
| BMI at delivery (Kg/cm$^2$) | 31.6±1.2 | 33.3±1.8 |
| Parity (number of gestations) | 1.9±0.3 | 1.8±0.3 |
| Gestational age at delivery (wk) | 39.1±0.3 | 39.0±0.2 |
| SBP (mmHg) | 120.3±2.6 | 124.5±2.8 |
| DBP (mmHg) | 76.2±2.7 | 74.6±2.5 |
| **Newborn** | | |
| Sex (male/female) | 8/4 | 6/6 |
| Weight (g) | 3384±102 | 3646±148 |
| Height (cm) | 49.0±0.3 | 49.0±0.4 |
| Cephalic perimeter (cm) | 34.2±0.2 | 35.1±0.3 |
| **Placenta** | | |
| Weight (g) | 545.8±38.5 | 603.6±43.1 |
| NBW/PIW | 6.6±0.4 | 6.4±0.4 |

BMI, body mass index. SBP, systolic blood pressure. DBP, diastolic blood pressure.
NBW, newborn weight. PIW, placental weight.





O18

## Over-expression of tumor-suppressor genes LDOC1, PARP1 and Caspase3 in Preeclamptic (PE) Placenta-derived Mesenchymal Stromal Cells (PDMSCs)

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**Objective:** A finely tuned programmed cell death is pivotal for normal human placental development. Aberrant cellular apoptosis may affect placental function resulting in placental diseases such as PE. We previously demonstrated that JunB, pro-apoptotic AP-1 family member acting in G1/S-phase cell cycle transition, was over-expressed in PE-PDMSCs, key structural component of placental villi. Herein we characterized the expression of LDOC1, PARP1 and Caspase 3, cell death modulators acting on G1/S and G2/M cell cycle phases respectively.

**Methods:** PDMSCs were isolated from control (n=30) and PE (n=30) placentae. At passage 5, control and PE-PDMSC were plated (1x10$^5$ cells/ml). Next, cells were collected and processed for mRNA isolation and cDNA preparation. PARP1, Caspase3 and LDOC1 gene expression were evaluated by Real Time PCR.

**Results:** We reported increased PARP1 (p=0.03), Caspase3 (p=0.04) and LDOC1 (p=0.05) gene levels in PE- relative to control PDMSCs. Since we previously described a parallel JunB over-expression in PE-PDMSCs, we next investigated a possible JunB-mediated regulation of LDOC1, PARP1 and Caspase3. To reach our goal, we performed JunB siRNA on control PDMSCs. JunB gene knock-down was accompanied by decreased LDOC1 (2.0 Fold Decrease) and increased Caspase3 (2.81 Fold Increase) mRNA levels. No effects were observed on PARP1 gene expression.

**Conclusion:** We demonstrated, for the first time to our knowledge, LDOC1, PARP1 and Caspase3 over-expression in PE-PDMSCs. Our data suggest that pathological PDMSCs are characterized by aberrant cell cycle regulation in both G1/S and G2/M phases. Moreover, the aberrant expression of these molecules in PE-PDMSCs could contribute to the impaired villous development typical of PE. Finally, JunB siRNA results indicated a JunB-mediated modulation of both LDOC1 and Caspase3. Indeed, LDOC1 down-regulation and Caspase3 up-regulation induced by JunB silencing could act as mechanism of cell death control in order to prevent excessive apoptosis and maintain cell homeostasis.

O19

## Human amnion epithelial cells mediate lung repair by influencing macrophage and regulatory T cell response

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**Background:** Human amnion epithelial cells (hAECs) have protective and reparative capabilities when administered immediately after and following established lung injury. hAECs can modulate macrophage response during repair by altering their polarity and function. However, the importance of other immune cell types in hAEC mediated lung repair is yet unknown.

**Hypothesis:** hAECs exert their reparative effects by inducing the maturation of naïve T cells into regulatory T cells (Tregs) and this step precedes the polarization of macrophages from proinflammatory M1 to proreparative M2.

**Methods:** We first determined the role of Tregs by treating bleomycin challenged *Foxp3-GFP knock in* mice with hAECs and measured the local Treg population by flow cytometry. The reliance of hAEC mediated lung repair on Tregs was further assessed by challenging $Rag1^{-/-}$ mice with bleomycin, followed by adoptive transfer of either Tregs or CF45+/FoxP3- cells. The extent of lung fibrosis and inflammation, and macrophage polarity and function were measured 7 and 14 days later.

**Results:** Administration of hAECs to bleomycin challenged *Foxp3-GFP knock in* mice induced Treg expansion in the lungs. Further, lung repair in bleomycin challenged Rag1-/- mice was most significant in the cohort of animals administered hAECs following adoptive transfer of Tregs. *In vitro,* hAECs directly induced FoxP3 transcription in naive CD4+ cells, primarily though TGF-β signaling. Additionally, hAEC mediated polarization of macrophages *in vivo* towards an M2 anti-inflammatory phenotype was only observed in animals that received hAECs as well as adoptively transferred Tregs.

**Conclusion:** Interaction between hAECs and Tregs contribute to their protective and reparative properties. Polarization of macrophages occurs as a consequence of this cellular interaction.





O20

## Placental telomere shortening in stillbirth: a sign of premature senescence?

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**Objective:** The objective of this study is to investigate placental telomere shortening in unexplained stillbirths (SB) as an indication of premature senescence and oxidative stress.

**Methods:** Placentas were collected from 42 unexplained SB (>22 weeks), 43 term and 15 preterm live births, at the Policlinico Hospital of Modena (Italy). DNA extracted from placentae were studied for telomere length by real time PCR. Standard curves were generated for telomere lengths from single copy gene amplifications using a reference DNA. The telomere length for each sample was derived based on the ratio of telomere length between the sample and single copy gene standard (T/S ratio).

**Results:** The mean ratio of placental telomere in normal term live births was 5.181 ±3.841. A twofold decrease in telomere length was seen in SBs (over all 2.455±1.239; $p < 0.001$). For early SB (above 34 weeks), the T/S was 2.8884±1.224 and for late SBs the T/S was 2.207±1.201, both lower than term live births (both $p < 0.01$). T/S remained lower both in small for gestational age-SB (2.639 ± 1.619) and appropriate for gestational age-SB (2.653 ± 1.335) with no difference between these subgroups (p = ns). Finally, T/S were significantly lower in SB compared with spontaneous preterm births (6.382± 5.525; $p < 0.01$). Specifically the highest results were in preterm with intact membranes (9.083± 5.664; < 0.01), whereas SB telomere length were similar to those of pPROM (3.296± 3.599; p = ns).

**Conclusions:** Substantial reduction in telomere length in SBs is indicative of placental senescence likely due to oxidative stress in response to specific risk exposure. These data provide mechanistic insights in cases of unexplained SBs where placental dysfunction due to premature ageing of placenta is likely an initiator of fetal demise.

O21

## Inflammation-induced surfactant protein A expression is mediated by pulmonary fibroblasts

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Intrauterine inflammation decreases the risk of respiratory distress syndrome in preterm infants, likely due to precocious surfactant production. The mechanisms mediating the effect of inflammation on surfactant production by the preterm lungs are unknown. We have previously shown that directly stimulating distal lung epithelial cells (DLECs) *in vitro*, with lipopolysaccharide (LPS; a pro-inflammatory stimulus), does not induce an increase in surfactant production. We hypothesised that inflammation acts via either macrophages or pulmonary fibroblasts to induce an increase in DLEC surfactant production. DLECs were isolated from fetal mice at E18.5 (n=6 litters/study). After 24 h in culture DLECs were either treated with conditioned media from (1) bone marrow-derived mouse macrophages; or (2) primary fetal mouse lung fibroblasts treated with either LPS- (1 μg/mL) or vehicle. Messenger RNA levels for the proinflammatory cytokine interleukin (IL)-1β, the chemokine CXCL1, and SP-A, -B, -C and –D were measured by qPCR 24 h after treatment. Conditioned media from LPS-treated macrophages increased IL-1β and CXCL1 mRNA levels in DLECs 63- ($p<0.05$) and 32-fold ($p<0.0005$), respectively, relative to media from vehicle-treated macrophages, but SP-A, -B, -C and -D mRNA levels were not affected by the treatment. Conditioned media from LPS-treated fibroblasts increased IL-1β and CXCL1 mRNA levels in DLECs 61- ($p<0.05$) and 40-fold ($p<0.005$), respectively, relative to media from vehicle-treated fibroblasts. Conditioned media from LPS-treated fibroblasts increased SP-A mRNA levels 10-fold ($p<0.005$), and SP-D mRNA levels doubled ($p<0.09$), compared to vehicle-treated fibroblasts. SP-B and -C mRNA levels were not significantly different between groups. A factor secreted by fetal pulmonary fibroblasts, in response to inflammation, increases fetal DLEC expression of SPs known to play a role in host defence in the lungs. These results begin to unravel the mechanisms whereby inflammation increases SP production to provide a respiratory benefit after preterm birth.





O22

## Effect of hypothyroidism on pancreatic β-cell mass and circulating insulin concentration in ovine fetuses

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**Introduction:** Thyroid hormones are important regulators of fetal growth, although their mechanism of action remains unclear. This study investigated the effect of hypothyroidism on pancreatic β-cell development in fetal sheep.

**Methods:** All procedures were carried out under the UK Animals (Scientific Procedures) Act 1986. Under general anaesthesia between 105-110 days of gestation (d; term~145d), one twin fetus was thyroidectomised (TX), while the other was sham-operated as a control (n=19 ewes). After maternal euthanasia, umbilical arterial blood was taken from both fetuses at either 129d or 143d (n=38). After fetal euthanasia, fetuses were weighed, measured and tissues collected. The whole fetal pancreas was weighed, fixed in 4% paraformaldehyde and embedded in paraffin wax. Pancreatic β-cells were identified immunohistochemically with an insulin antibody and their mass was calculated using the Cavalieri estimator (Visiopharm, Denmark). Plasma insulin, triiodothyronine ($T_3$) and thyroxine ($T_4$) concentrations were measured by ELISA or RIA. Data (mean ± SEM) were assessed by two-way ANOVA followed by Tukey's *post hoc* test.

**Results:** In TX fetuses, plasma $T_3$ and $T_4$ concentrations were at the lower limit of assay detection ($T_3$, 6.7pg/ml; $T_4$, 7.6ng/ml) at both ages. There were no differences in absolute body or pancreas weight between TX and sham fetuses at either age. At 143d, limb lengths, lungs, heart, stomach and small intestine of TX fetuses were growth retarded, while the kidneys and perirenal adipose tissue were significantly enlarged, compared with sham fetuses ($p<0.05$).
Plasma insulin levels were significantly higher in TX fetuses at both 129d (sham: 92±60ng/L; TX: 129±33ng/L; $p<0.05$) and 143d (sham: 56±10ng/L; TX: 168±24ng/L; $p<0.05$). Relative β-cell mass to body weight was significantly greater in TX fetuses at both 129d (sham: 47±4mg/kg; TX: 79±6mg/kg; $p<0.05$) and 143d (sham: 46±7mg/kg; TX: 75±10mg/kg; $p<0.05$).

**Conclusions:** The results indicate that there are interactions between thyroid hormones and insulin in regulating fetal growth during hypothyroidism.

*Funded by* the BBSRC and Nigel Groome PhD studentship.

O23

## Exhaled CO2 measurement in ventilated infants

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**Background:** The rate of $CO_2$ exhalation is mostly determined by the rate of $CO_2$ production in steady state. $CO_2$ production rate, on the other hand, is determined by a status of metabolism ($O_2$ consumption rate and respiratory quotient). Assessment of $CO_2$ producing status is important and useful in the management of newborn infants.

**Objectives:** To study $CO_2$ exhalation rate and its changes over time in the neonatal and early infantile period in intubated infants on a ventilator.

**Methods:** Subjects were intubated infants on a ventilator due to diffuse respiratory disease in the NICU of Tokai University Hospital during the period Oct 2012-Mar 2013. They were oro-tracheally intubated with size 2.5-3.5mmID tracheal tube. $CO_2$ exhalation rate was measured using NICO7300 monitor (Novametrix®) under calm and stable condition.

**Results:** Thirty four measurements were performed on post-natal day 0-19 (median 2) in 18 infants. Their gestational age ranged 25-38 (median 34) weeks and birth weight 610-3700 (median 2360) grams. $CO_2$ exhalation rate (V' $CO_2$) was 0.4-4.9mL/min/kg. Excluding 10 measurements where infants were intubated with 2.5mmID ETT (under which condition, large ETT leaks can make exhaled $CO_2$ measurement inaccurate), V'$CO_2$ was 0.6~4.6mL/min/kg and showed trends of increasing V'$CO_2$ according to increasing post-natal days (PND) (V'$CO_2$=0.23PND+2.22; $r^2$=0.251, p=0.014).

**Conclusions:** CO2 production rate showed increasing trends over time after birth. This is a point to be considered in the respiratory management of newborn infants.





O24

## Controlling contractions in human myometrium, an eye on channels

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**Introduction:** Human myometrial contractions are controlled by calcium influx through voltage-gated calcium channels during the action potential (AP), hence membrane voltage is essential in determining contractility. Thus, there has been much interest in the processes that set membrane voltage. Our aim was to identify the mechanisms involved in order to better target and combat inappropriate uterine contraction, such as those that occur before term or during failure to progress in term labour.

**Methods:** Myometrium was obtained from consenting women at term, before or after labour onset. Membrane voltage was recorded simultaneously with contractions in strips, ion channel activity was recorded in acutely isolated myometrial smooth muscle cells using patch clamp technology and protein levels were analysed using Western blotting.

**Results:** The level of negativity of the membrane potential was resistant to blockade of most classes of potassium channels. Myometrium from obese women remained unusually negative at term and this suppressed APs and contraction. Depolarization of these tissues occurred upon blockade of two-pore potassium channels. Interestingly, membrane voltage in human myometrium was exquisitely sensitive to blockade of the Na/K ATPase pump, especially in tissues from obese women. We also identified two potassium channels that were without effects on setting membrane voltage but which, when blocked, resulted in significant increases in the AP and contraction. Blockade of big-conductance, calcium-activated potassium ($BK_{Ca}$) channels significantly increased the amplitude of the AP, while ether-a-go-go potassium channel blockade increased AP duration.

**Conclusions:** Membrane voltage and contraction in human myometrium is set predominantly by two-pore potassium channels and the Na/K pump, and manipulating these restored membrane voltage values and contraction in quiescent tissues obtained from obese women with failure to progress in labour. Of critical importance, this was achieved while retaining the ability of the tissues to fully relax between contractions, essential for survival of the fetus.

# ORAL COMMUNICATIONS

## Oral Session IV - Fetal & Neonatal brain (I)

***Chairs***
***Graham Jenkin*** *(Monash University, Australia)*
***Emilio Herrera*** *(University of Chile, Chile)*





O25

## Synergistic white matter protection with acute-on-chronic endotoxin and subsequent asphyxia in preterm fetal sheep

Bennet Laura [1], Van den Heuij Lotte [1], Davidson Joanne, Mathai Sam [1], Lear Chris [1], Booth Lindsea [1], Drury Paul [1], Fraser Mhoyra [1], Gunn Alistair Jan [1]

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**Background:** Perinatal asphyxia and exposure to intrauterine infection are associated with impaired neurodevelopment in preterm infants. Acute exposure to non-injurious infection/inflammation can either protect or sensitize the brain to subsequent hypoxia-ischemia. However, the effects of subacute infection/inflammation are unclear. In this study we tested the hypothesis that acute-on-chronic exposure to lipopolysaccharide (LPS) would exacerbate white matter injury after subsequent asphyxia in preterm fetal sheep.

**Methods:** Fetal sheep at 0.7 of gestational age received a continuous LPS infusion at 100 ng/kg for 24 hours, then 250 ng/kg/24 hours for 96 hours, plus 3 x1 µg boluses of LPS at 48, 72 and 96 hours or the same volume of saline. 4 hours after the last bolus complete umbilical cord occlusion or sham occlusion was induced for 15 minutes. Sheep were killed 10 days after the start of infusions.

**Results:** LPS exposure was associated with induction of microglia and astrocytes and loss of total and immature/mature oligodendrocytes (n = 9) compared to sham controls (n = 9). Umbilical cord occlusion with saline infusions was associated with induction of microglia, astrogliosis and loss of immature/mature oligodendrocytes (n = 9). LPS exposure before asphyxia (n = 8) was associated with significantly reduced microglial activation and astrogliosis and improved numbers of immature/mature oligodendrocytes compared to either LPS exposure or asphyxia alone.

**Conclusions:** Contrary to our initial hypothesis, the combination of acute-on-chronic LPS with subsequent asphyxia reduced neuroinflammation and white matter injury compared with either intervention alone.

O26

## The effect of customized versus population standards on the identification of small for gestational age in a cohort of neonates who had intra-partum acute-distress

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**Objective:** To evaluate if the application of customized standards may reveal cases of small for gestational (SGA) babies that were undiagnosed using population standards in a cohort of neonates delivered by cesarean section performed for acute fetal distress.

**Methods:** Retrospective study on singleton pregnancies delivered by cesarean section for fetal distress after 34 weeks of gestation in a third level hospital. Maternal characteristics and gestational age, sex, and weight of newborns were reviewed. Birth weight (BW) percentile was calculated applying the population and customized standards. SGA was defined as a BW <10$^{th}$ percentile. A chi-square test was used to compare the rate of SGA according to each classification. Cohen's kappa coefficient was used to test the agreement between the two classifications.

**Results:** Seventy-six cases of cesarean section performed for acute fetal distress were identified. Most of patients were European (n=67, 88%) and 75% had a normal pre-pregnancy BMI. Sixteen percent of patients were overweight and only one was obese. By applying the population-based standards, 13 babies (17%) were classified as SGA, while the application of customized standards identified 17 SGA babies (22%). The difference was not statistically significant (p=0.41). In 10 cases the two standards were concordant in establish the diagnosis of SGA, while in 10 cases they were discordant: 3 cases (4%) were SGA based on population but not customized standards and 7 cases (9%) were SGA based on customized but not on population reference (p=0.34). The concordance between the two classification methods was moderate (Cohen's kappa 0.59, p<0.001).

**Conclusion:** Customized assessment identified a larger number of SGA compared to population standards, but the difference was not statistically significant. The low variety observed in our population in terms of ethnicity and the high prevalence of normal-weight patients may explain why the two classifications showed a significant concordance.

| Maternal demographics (n=76) | |
|---|---|
| **Ethnicity:** | |
| European | 67 (88%) |
| East-Asian | 5 (7%) |
| Other | 4 (5%) |
| **BMI (kg/m$^2$)** | |
| <18.5 | 6 (8%) |
| 18.5-24.9 | 57 (75%) |
| 25-29.9 | 12 (16%) |
| ≥ 30 | 1 (1%) |

| SGA neonates | | |
|---|---|---|
| **Population standards** | **Customized standards** | p |
| 13 (17%) | 17 (22%) | 0.41 |





O27

## Peripheral myeloid cells invade the brain following neonatal hypoxia-ischemia

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CNS inflammation is believed to be an important factor in the pathophysiology of hypoxic-ischemic (HI) injury in the developing brain. The traditional view of an "immune privileged" CNS is progressively giving way to the concept of an "immune specialized" CNS, which actively interacts with the periphery. However, the contribution of peripheral immune cells to central inflammation remains unclear.

*Lys*-EGFP-*ki* mice, a strain expressing EGFP in peripheral myeloid cells, but not in microglia, were used to investigate the influx of peripheral immune cells following injury in a murine model of neonatal hypoxia-ischemia (HI). To induce HI the left common carotid artery was permanently ligated in postnatal day 9 mice, followed by exposure to 50 minutes of hypoxia (10%). The presence of peripheral myeloid cells in the CNS was investigated using FACS analysis and by immunohistochemistry staining from 24 hours to 86 days post-HI. Invading myeloid cells were identified in tissue sections through their expression of EGFP and classified by FACS analysis as infiltrating monocyte/macrophages (CD11b+EGFP+Ly6G-) and infiltrating granulocytes (CD11b+EGFP+Ly6G+).

We observed a progressive accumulation of EGFP positive peripheral myeloid cells in the ipsilateral hemisphere from 2 hours after HI, with the earliest expression found in CD31+ blood vessels, but later cells were identified in the parenchyma. FACS analysis demonstrated infiltration of both hematogenous monocyte/macrophages (CD11b+EGFP+Ly6G-) and granulocytes (CD11b+EGFP+Ly6G+) in response to HI, particularly at 3-14 days after HI.

Peripheral myeloid cells invade the CNS following HI. These cells progressively accumulate in the ipsilateral thalamus, hippocampus, and cortex where they persist for several weeks post-insult. The myeloid cell infiltrate represents a heterogeneous population of hematogenous monocyte/macrophages and, to a lesser extent, granulocytes.

O28

## Perinatal asphyxia leads to region specific overexpression of PARP-1, increasing the expression of pro-inflammatory cytokines and cell death: prevention by systemic nicotinamide treatment

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Perinatal asphyxia (PA) is a leading cause of neuronal damage in newborns, resulting in long-term neurological and cognitive deficits, in part due to impairment of neurocircuitries of the basal ganglia and hippocampus, which are particularly vulnerable to PA. The insult can be as severe as to menace the integrity of the genome, triggering the overactivation of sentinel proteins, including poly (ADP-ribose) polymerase-1 (PARP-1). PARP-1 overactivation implies increased energy demands, worsening the energy failure, depleting further $NAD^+$ availability.

Using a rat model of global PA, we report here evidence that PA increases PARP-1 activity, short after the insult, triggering a signalling cascade leading to nuclear translocation of the NF-kB subunit p65, modulating the expression of the inflammatory related molecules, IL-1β and TNF-α, increasing apoptotic-like cell death, monitored in mesencephalon, telencephalon and hippocampus 0-24h after the insult. PARP-1 activity was increased following PA in all explored brain tissue, but the NF-ϰB signalling pathway was only activated in mesencephalon and hippocampus, regions where apoptotic-like cell death was significantly increased at 24h, compared to that observed in the corresponding controls. A single dose of the PARP-1 inhibitor nicotinamide (0.8 mmol/kg, i.p.) 1h post delivery prevented the effect of PA on PARP-1 activity, p65 translocation and expression of pro-inflammatory cytokines and apoptotic-like cell death.

Thus, the present results show that perinatal asphyxia leads to region specific overexpression of PARP-1, increasing also the expression of pro-inflammatory cytokines and cell death, effects prevented by systemic neonatal nicotinamide administration.

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O29

## Status epilepticus after prolonged umbilical cord occlusion is associated with greater neural injury fetal sheep at term-equivalent

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The majority of pre-clinical studies of hypoxic-ischemic encephalopathy at term-equivalent have focused on either relatively mild insults, or on functional paradigms of cerebral ischemia or hypoxia-ischemia/hypotension. There is surprisingly little information on the responses to severe fetal asphyxia. In this study we examined the evolution and pattern of neural injury after prolonged umbilical cord occlusion (UCO). 36 chronically instrumented fetal sheep at 125–129 days gestational age (term = 147 days) were subjected to either UCO until mean arterial pressure was < = 8 mmHg (n = 29), or sham occlusion (n = 7). Surviving fetuses were killed after 72 hours for histopathologic assessment with acid-fuchsin thionine, NeuN, Olig-2, Iba-1 and caspase-3. After UCO, 11 fetuses died with intractable hypotension and 5 ewes entered labor and were euthanized. The remaining 13 fetuses showed marked EEG suppression followed by evolving seizures starting at 5.8 (6.8) hours (median (interquartile range)). 6 of 13 developed status epilepticus, which was associated with a transient secondary increase in cortical impedance (a measure of cytotoxic edema, $p<0.05$). All fetuses showed moderate to severe neuronal loss in the hippocampus and the basal ganglia but mild cortical cell loss ($p<0.05$ vs sham occlusion) as assessed with acid-fuchsin thionine. Status epilepticus was associated with more severe terminal hypotension ($p<0.05$) and subsequently, greater neuronal loss ($p<0.05$). In conclusion, profound UCO in term-equivalent fetal sheep was associated with delayed seizures, secondary cytotoxic edema, and subcortical injury, consistent with the predominant pattern after peripartum sentinel events at term. It is unclear whether status epilepticus exacerbated cortical injury or was simply a reflection of more severe hypotension and hypoperfusion during asphyxia.

O30

## Suppression of neurosteroid synthesis has adverse long-term effects on outcomes

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Neurosteroids have important roles in fetal brain development. The suppression of levels of the key neurosteroid, allopregnanolone using the 5a-reductase inhibitor, finasteride, during late gestation results in increased apoptosis, disrupted oligodentrocyte development and markedly reduced myelination in the fetal brain at term. However, less is known regarding the long-term consequences of these low neurosteroid levels on behavioural outcomes. The aim of this study was to investigate the effect of suppression of neurosteroid production during pregnancy on behavioural outcomes in guinea pigs at 7 days of age. For these studies, time-mated, outbred pregnant guinea pig dams received vehicle (45% b-cyclodextrin) or finasteride (25mg/kg) once daily, commencing at GA60 until delivery (term ~71 days). At postnatal day (PND) 7 all pups underwent behavioural testing using open field (OF) and novel object recognition tests (NORT). After testing, brains were collected and analysed for myelin basic protein (MBP) area coverage in lobes VIII and X, and the deep white matter of the cerebellum. The female offspring from pregnancies treated with finasteride spent less time exploring the inner zone of the open field compared to those from vehicle treated pregnancies ($p=0.005$), whereas males were not affected. Finasteride-exposed animals appeared to spend less time exploring objects in NORT, with females also appearing to spend less time investigating a new vs. familiar object, however these trends were not significant ($p=0.09$). No differences were found between treatments for MBP coverage area in the cerebellum. These results indicate that there is “catch-up” development of myelination (MBP imunostaining) in finasteride exposed offspring by PND 7 compared to term. The reduced neurosteroid environment *in utero,* however, had long-term effects on offspring, with females showing a more anxious phenotype following finasteride exposure. These observations suggest low neurosteroid levels during pregnancy have ongoing adverse effects on behavioural outcome.





O31

## The impact of antenatal steroids and magnesium sulphate on neurotrophic factors in preterm infants

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**Background:** Up to 50% of preterm children will experience problems with neurodevelopment by school age. Antenatal steroids and magnesium sulphate ($MgSO_4$) administered to women in threatened preterm labour are neuroprotective. Neurotrophins are proteins involved in neural growth, survival and differentiation, and are important for development of the nervous system. The impact of antenatal steroids and $MgSO_4$ on neurotrophins remains unknown.

**Aims:** The aim was to assess levels of brain derived neurotrophic factor (BDNF), neurotrophic factor-3 (NT3), neurotrophic factor-4 (NT4), nerve growth factor (NGF) and reelin, according to gestational age, exposure to $MgSO_4$ and steroid therapy.

**Methods:** Cord blood from 266 preterm infants (<37 weeks) was collected at delivery and BDNF, NT3, NT4, NGF and reelin levels were measured by ELISA. The impact of $MgSO_4$ was assessed only in infants born ≤30 weeks gestation (n=60).

**Results:** NT3, NT4 and BDNF increased across the preterm period ($p<0.001$). NGF was higher in males ($p=0.031$) while reelin was higher in females, but only during the late preterm period (33-36 weeks, $p=0.015$). In infants ≤ 32 weeks, steroid exposure did not affect neurotrophins. In late preterm infants, NT3 levels were higher in unexposed infants and those born within 24 hours of steroid exposure, compared to those born outside of 24 hours ($p<0.001$), while reelin was higher when born within 24 hours of steroid exposure. BDNF, NGF and NT4 were unaffected by steroids in late preterm infants. In infants born <30 weeks, an interaction effect was observed between $MgSO_4$ and steroids on BDNF ($p=0.003$). BDNF increased within 24 hours of steroid exposure ($p<0.01$) and returned to baseline thereafter only in infants exposed to $MgSO_4$. A similar trend was observed in NT3 levels, with no effect of $MgSO_4$ on NT4, NGF or reelin.
**Conclusions:** The transient increase in BDNF and NT3 following both $MgSO_4$ and antenatal steroid therapies, which are not observed following either therapy alone, may alter neurodevelopmental outcomes in preterm infants. The alterations in NT3 and reelin in late preterm infants following steroid therapy warrants further investigation

O32

## Progesterone treatment of the growth restricted fetus - a natural therapy

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IUGR fetuses are likely to be born preterm, and therefore the mother usually receives glucocorticoids such as betamethasone to promote fetal lung maturation. We have however shown that antenatal betamethasone significantly decreases the essential neurosteroid allopregnanolone (AP) in the fetal brain and causes cellular injury (Yawno et al, 2014). In this study of IUGR fetal sheep, we examined if exogenous progesterone - the natural substrate for AP synthesis - is able to maintain fetal brain AP and reduce brain injury following maternal betamethasone treatment.

Single umbilical artery ligation (SUAL) was performed to induce IUGR at 103 days gestation in fetal sheep. Ewes were given betamethasone (or saline) on days 10 and 11 after surgery, and progesterone (or vehicle) on days 9, 10 and 11. After euthanasia on day 124, fetal brains were collected to determine AP and progesterone levels and brain histopathology.

In IUGR fetuses, betamethasone treatment reduced fetal brain AP concentration (1.3 ± 0.6 pmol/ml) compared to control+saline animals (5.1 ± 1.2 pmol/ml; $p<0.05$), while progesterone treatment prevented this decrease (5.2 ± 0.6 pmol/ml). Whereas betamethasone treatment increased the number of iba-$1^+$ inflammatory cells in fetal brain white matter (685 ± 180 vs [saline control] 279 ± 98 cells/$mm^2$), this increase was significantly reduced in the progesterone+BM group (300 ± 122 cells/$mm^2$). Progesterone treatment also reduced the number of apoptotic cells (caspase-$3^+$) and improved myelination (CNPase$^+$ staining) compared to non-progesterone treated animals.

This study confirms that some of the deleterious effects of maternally-administered glucocorticoids on the developing brain arise because of suppression of endogenous AP synthesis. We show that co-treatment with progesterone prevents the loss of this essential neurosteroid, and ameliorates brain injury following betamethasone in IUGR fetuses. Progesterone may be an effective adjuvant neuroprotective therapy that can be used clinically when preterm birth is likely to occur.

Yawno et al (2014) Neuropharmacology.





# ORAL COMMUNICATIONS

Oral Session V - Miscellaneous

***Chairs***
***Tomoaki Ikeda*** *(Mie University Graduate School of Medicine, Japan)*
***Alessandro Rolfo*** *(University of Turin, Italy)*

O33

## The effect of Holder pasteurization on human milk glycosaminoglycans

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**Objectives:** The benefits of human milk for preterm infants are mainly due to its nutritional characteristics and to the presence of biologically active compounds. Among these compounds, glycosaminoglycans play an emerging leading role. When mother's milk is unavailable or in short supply, pasteurized donor milk represents an important nutritional alternative. The aim of this study was to evaluate the effect of Holder pasteurization on the concentration of different glycosaminoglycans in preterm human milk.

**Methods:** Milk samples collected from nine mothers having delivered preterm were divided into two parts. One part of each sample was immediately frozen (-80 °C), while the other part was pasteurized with the Holder method before being frozen at - 80 °C. Specific analytical procedures were applied to evaluate the amount, composition and structure of main human milk glycosaminoglycans.

**Results:** No significative differences were measured between not-treated and pasteurized samples for total glycosaminoglycans content, relative percentages of chondroitin sulphate and heparan sulphate and main parameters related to galactosaminoglycans structure, even if a slight decrease of total glycosaminoglycans content of ~18% was observed in treated samples.

**Conclusion:** Our results indicate that the Holder pasteurization does not substantially affect the concentration of the main human milk glycosaminoglycans.





O34

## Low molecular weight heparin modulates d6 decoy receptor expression in preeclamptic human umbilical vein endothelial cells

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**Objectives:** Preeclampsia (PE) is a pregnancy-related syndrome characterized by exacerbated placental-maternal endothelial inflammatory response in which pro-inflammatory chemokines play a crucial role. Their biological effects are inhibited by D6 decoy receptor, which functions as a scavanger due to lack of sequence motifs important for signal transduction.
It is well known that low molecular weight heparin (LMWH) are used during pregnancy since several trials have demonstrated their efficacy in treating systemic inflammation correlated to pregnancy complication such as PE. Nevertheless, LMWH molecular mechanisms are still unclear. In this study, we investigated D6 expression in human umbilical vein endothelial cells (HUVECs) derived from normal and PE placentae after LMWH treatment, in order to better understand D6 role in inflammation typical of PE and LMWH effect on the placental endothelium.

**Methods:** Primary endothelial cells were isolated from umbilical cord biopsies collected from PE (n=10) and control (CTRL, n=10) placentae immediately after delivery. Cells were cultured as follow: 1) plain culture medium; 2) medium supplemented by LMWH (5 units, Parnaparin, Alfa Wassermann, Italy). D6 protein levels were determined by Western Blot analysis. Data were collected at 24, 48 and 72 hours.

**Results:** Western blot assays showed two bands of 46 kDa and 49 kDa, corresponding to the intracellular (non-glycosylated) and plasma membrane (glycosylated) forms of D6 receptor respectively. There was an increase in both 49 and 46 kDa D6 forms in HUVECs treated by LMWH relative to CTRL at 24h and 48h (24h: 2,1 and 1.63 Fold Increase; 48h: 2,3 and 1.3 Fold Increase, respectively). No differences were found at 72h between HUVECs treated with LMWH and CTRL.

**Conclusions:** Our data suggest an LMWH anti-inflammatory activity on the placental endothelium through, D6 modulation. LMWH could promote D6 migration from intracellular stores to the plasma membrane, thus enhancing its scavenger activity. Further investigations are required.

O35

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

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**Background:** Human amnion epithelial cells (hAECs) are anti-inflammatory, and can modulate the effects on lung and brain development of experimental intrauterine inflammation in preterm fetal sheep.

**Aims/Hypothesis:** We aimed to determine the effect of hAECs on the acute fetal inflammatory response to intra-amniotic injection of lipopolysaccharide (LPS).

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

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

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

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

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





O36

## Effects of prenatal hypoxia on cardiac function over ageing

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**Introduction:** Ageing (1) as well as prenatal hypoxia (2-4) are established risk factors for increasing susceptibility to cardiovascular disease. However, their inter-relationship has been little explored. Here, we investigated in rats the effects of prenatal hypoxia on cardiac function in adult and ageing rats at 4 and 15 months of age.

**Materials and Methods:** Female Wistar rats were randomly divided into normoxic (N: 21% $O_2$) or hypoxic (H: 14% $O_2$) pregnancy from days 6-20 of gestation. This experimental model of hypoxia does not affect maternal food intake. At birth, litters were culled to 8 pups (5 males and 3 females) and weighed weekly. At 4 and 15 months, following euthanasia, hearts were isolated from 1 male offspring per litter and cardiac function was investigated in a Langendorff preparation. Cardiac mitochondrial oxygen consumption was determined by permeabilised cardiac muscle fibre respirometry in another 1 male offspring per litter.

**Results and Discussion:** In offspring from normoxic pregnancy, ageing impaired diastolic dysfunction (Fig. 1a), decreased coronary flow rate (Fig. 1b) and limited myocardial mitochondrial oxygen consumption (Fig. 1c). Offspring of hypoxic pregnancy showed accelerated cardiac ageing already at 4 months of age (Fig. 1 a-c).

**Conclusions:** Ageing impairs cardiac diastolic and mitochondrial function. Prenatal hypoxia accelerates the cardiac ageing process. A component of the increased susceptibility to cardiovascular disease in offspring from hypoxic pregnancy may be due to accelerated ageing.

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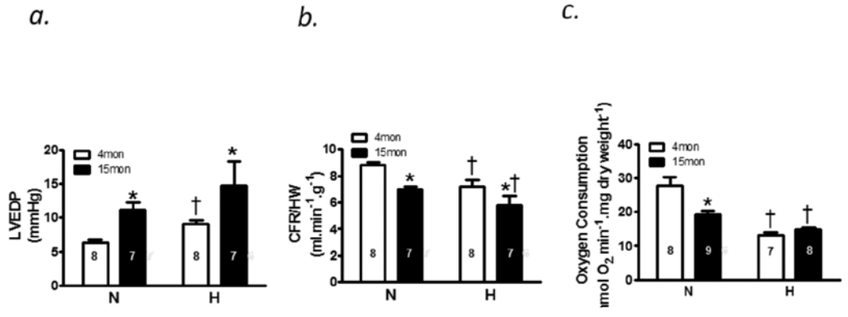


Figure 1. Values are mean ± S.E.M. N, normoxic; H, hypoxic; LVEDP; left ventricular end diastolic pressure; CFR, coronary flow rate; HW, heart weight. Significant difference ($P<0.05$): * vs. 4 month, † vs. N, two way ANOVA + Tukey test.

O37

## Do deficits in Trop2 lead to reduced cerebellar granule cell migration in growth-restricted offspring?

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Intrauterine growth restriction (IUGR) is a major cause of antenatal brain injury leading to neurodevelopmental sequelae including cerebral palsy (CP). We have previously shown that IUGR is associated with impaired migration of cerebellar granule cells[1]. Here we investigate whether deficits in Trop2, a known regulator of cell migration[2], are associated with altered cerebellar development in IUGR. Pregnant WKY rats underwent bilateral uterine vessel ligation (Restricted; n=18 litters) to induce IUGR at embryonic (E) day 18 (E18; term=E22); controls underwent sham surgery (Control; n=17 litters). Cerebellums were collected at E20, postnatal day (PN) 2, 7, 14 and 35. Trop2 mRNA expression was assessed in controls at each age. In Restricted compared to Control pups (PN7, PN35) Trop2 expression, TROP2-immunostaining and cerebellar structure were assessed. In Controls, Trop2 mRNA levels were highest at PN7, when the peak period of granule cell migration begins[3] and reduced by PN35, when granule cell migration is complete[3]. At PN7, TROP2-immunofluorescence was localised to granule cells within the external granule layer (EGL), molecular layer (ML) and internal granule layer (IGL) and persisted in the IGL at PN35. At PN7 in Restricted versus Controls, Trop2 expression decreased by ~90% (p=0.02) and TROP2 protein was decreased in the EGL and IGL (p = 0.03). The EGL width was increased (p=0.007) in PN7 Restricted pups versus Controls, suggesting delayed granule cell migration from the EGL. At PN7 there was no change in the width of the proliferative zone of the EGL or the density of the Bergmann glial fibres in Restricted vs. Control pups. Trop2 may be a novel mechanism regulating cerebellar granule cell migration. Deficits in Trop2 may subsequently affect cerebellar circuitry and long-term functioning following IUGR in CP children.

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O38

## Differences in heart structure of lambs immediately after preterm and term birth

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Preterm birth (<37 weeks gestation) occurs in 9-12% of all births; the majority are moderately preterm (32-36 weeks gestation). We proposed that the myocardium of a preterm heart is immature and ill-prepared for the haemodynamic transition at birth. We aimed to compare cardiac muscle structure and cardiomyocyte growth in moderately preterm and term lambs in the immediate period after birth.

Pregnant ewes were induced to deliver vaginally at 131±1 days of gestation (moderately preterm) or at term (≈148 days). Clinically relevant doses of Betamethasone were administered 24h and 48h before birth to the preterm cohort. Two days after birth, lambs were humanely killed and hearts excised, weighed and perfusion-fixed. The left and right ventricles (LV,RV) were sectioned transversely (10mm slices) and ventricular wall thickness determined using image analysis. Ventricular wall and chamber volumes and the number of cardiomyocyte nuclei in the LV and adjoining septum (S) were stereologically determined.

Preterm lambs were significantly lighter at birth and necropsy compared to term lambs. Two days after birth, preterm lambs had thinner walls of the RV (P=0.002), LV (P=0.001) and septum (P=0.03); however, when adjusted for body weight, RV, LV and septal thicknesses were greater (P<0.0001). Additionally, wall volume of the RV and LV+S was lower (P<0.0001) in preterm hearts but there were no differences relative to body weight. Preterm lambs also exhibited smaller RV (P<0.0001) and LV (P=0.002) chamber volumes and relative RV chamber volume (P=0.029). There were significantly fewer (P=0.003) cardiomyocyte nuclei in the LV+S of preterm lambs than in term lambs.

**Conclusions:** The observed reduction in the number of cardiomyocyte nuclei in the LV+S is indicative of reduced total cardiomyocyte number and/or altered nuclearity. Differences in cardiac structure in the moderately preterm heart likely render it vulnerable to the haemodynamic transition at birth, and may have life-long cardiovascular consequences.

O39

## Human amniotic epithelial cells: chronic effects on evolving brain injury in preterm fetal sheep

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**Introduction:** Brain injury as a result of perinatal asphyxia is highly prevalent amongst preterm infants, however at present there are no effective treatments available. The literature suggests that stem cell therapy may offer neuroprotection. Human amniotic epithelail cells (hAECs) are effective in reducing inflammation and improve functional outcomes in a number of different models of brain ischemia and stroke. In this study we investigated the effects of bolus intranasal administration of hAECs on the progression of injury in preterm fetal sheep post asphyxic insult.

**Methods:** In preterm fetal sheep, blood pressure, heart rate and EEG activity were continuously monitored before, during, and for 21 days after asphyxia induced by 25 min umbilical cord occlusion (UCO) at 103/104 days gestation. An intranasal (IN) bolus of either vehicle or hAECs ($30*10^6$ cells/2ml) was given at 1 d, 3 d and 10 d post-UCO. Fetal brains were histologically assessed for injury and evidence of cell migration.

**Results:** hAECs were still present and alive in the brain parenchyma at 21 days post UCO. As expected there were no differences in physiological parameters or seizures during the experiment. No differences in number of olig-2 positive oligodendrocytes were found between groups in the periventricular and intragyral white matter. There was a significant difference in CNPase positive late oligodendrocytes progenitor cells in these brain areas. Deap gray matter is still being evaluated.

**Conclusion:** Improvement in the ratio of CNPase positive late OPCs and olig-2 positive oligodendrocytes suggests that hAECs may reduce the maturational arrest after asphyxia in the periventricular and intragyral white matter. Thus, repeated doses of hAECs via intranasal administration improve preterm fetal white matter maturation after a hypoxic insult.





O40

## The effect of increased placental vascular resistance and hypoxemia on fetal left ventricular myocardial performance index

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**Objectives:** We investigated the effect of increased placental vascular resistance on fetal left ventricular myocardial performance index (MPI). Furthermore, we assessed whether hypoxemia affects divergently left ventricular MPI in fetuses with or without increased placental vascular resistance.

**Methods:** Data from 24 chronically instrumented anesthetized and mechanically ventilated pregnant sheep at 115–129 days of gestation were used for this report. 12 ewe fetuses underwent embolization of the placental vascular bed to increase its vascular resistance. Fetal left ventricular MPI was measured using pulsed Doppler technique at the beginning of the experiment in embolized as well as non-embolized fetuses. Maternal and fetal hypoxemia (defined as maternal oxyhemoglobin saturation of 80–90%) was induced by replacing oxygen by medical air in the rebreathing circuit, and the left ventricular MPI was measured again.

**Results:** Umbilical artery PI was significantly increased and combined cardiac output decreased in the embolized fetuses (1.22±0.28 vs. 0.78±0.15, and 1297±405 ml/min vs. 1871±695 ml/min). No significant difference was observed in the left ventricular MPI between the two groups. Hypoxemia was associated with a marked increase in the left ventricular MPI of non-embolized fetuses (0.34±0.06 v/s 0.46±0.10, $p < 0.005$). The increase in MPI was more modest in embolized fetuses (0.36±0.07 v/s 0.41±0.07), but was still statistically significant ($p = 0.036$).

**Conclusions:** Fetal hypoxemia is associated with a significant increase in left ventricular MPI. Increased placental vascular resistance does not seem to modify this response.

# ORAL COMMUNICATIONS

Oral Session VI - Fetal & Neonatal brain (II)

***Chairs***
***Tullia Todros*** *(University of Turin, Italy)*
***Dan Rurak*** *(University of British Columbia, Canada)*





O41

## Cerebral oxygenation deficits are assocaited with persistent periodic breathing in preterm infants after hospital discharge

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**Background:** Periodic breathing is common in preterm infants, but is usually resolved by term equivalent age. The aim of our study was to assess the incidence and impact of periodic breathing on oxygen saturation ($SpO_2$) and brain tissue oxygenation index (TOI) over the first 6 months after term equivalent age.

**Method:** Twenty-four preterm infants (27-36 weeks gestational age) were studied with daytime polysomnography. Recordings were made in both quiet sleep (QS) and active sleep (AS) and in both the prone and supine positions at 2-4 weeks, 2-3 months and 5-6 months term corrected age. $SpO_2$ and TOI (NIRO-200 spectrophotometer) were recorded. Periodic breathing episodes were defined as ≥3 sequential apneas lasting > 3s.

**Results:** A total 164 individual episodes of periodic breathing were recorded in 19 infants at 2-4 weeks, 62 in 12 infants at 2-3 months and 35 in 10 infants at 5-6 months. Only 2 infants did not exhibit periodic breathing. There was no effect of gestational age on periodic breathing frequency. duration and $SpO_2$ nadir during episodes were not affected by postnatal age in either sleep state or position, however the nadir in TOI increased in AS from a mean of -7% to -12% in both positions ($p<0.05$).

**Conclusions:** We found that the majority of preterm infants discharged home without clinical respiratory problems had persistent periodic breathing. Although in most infants periodic breathing was not associated with significant falls in $SpO_2$ or TOI, several infants did have significant desaturations and reduced cerebral oxygenation especially during AS, suggesting that routine oximetry before discharge may be warranted.

O42

## Exploring umbilical cord blood stem cell populations for the prevention of cerebral palsy

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**Background:** Cerebral Palsy (CP) is the most prevalent cause of chronic disability in children. In 2012, the Cerebral Palsy Alliance reported that 200 families sought overseas stem cell treatment for their child with cerebral palsy. However, it remains unknown if such treatments are effective. In this study we examined whether umbilical cord blood (UCB) mononuclear cells, which contain different stem cells including endothelial progenitor cells (EPCs) and mesenchymal stromal cells (MSCs), could hold the key to neuronal repair and regeneration after injury.

**Methods:** UCB mononuclear cells were obtained at delivery from term lambs. Cells were cultured and cell characteristics were investigated by flow cytometry and immunohistochemistry. For in vivo studies, culture expanded UCB cells were administered 12h after birth asphyxia and lambs monitored regularly. At 72h magnetic resonance spectroscopy (MRS) was performed followed by post-mortem.

**Results:** Flow cytometric analysis of culture expanded UCB cells revealed a distinct population of CD45+ and CD45- cells. CD45+ cells were capable of acetylated-LDL uptake and expressed VEGFR2, both characteristics of EPCs. Cultured UCB cells were also able to differentiate into adipocytes, osteocytes and myocytes, which suggests the presence of MSCs. MRS revealed an increase in neuronal integrity in asphyxic lambs that received culture expanded UCB cells at 12h and UCB cells were found throughout the brain in 1/4 lambs. IL-10, an anti-inflammatory cytokine, was also increased in the CSF of UCB treated lambs.

**Conclusion:** Two important cells populations, EPCs and MSCs, can be detected in sheep cord blood and administration of culture expanded UCB cells improved neuronal integrity and decreased inflammation in term asphyxiated lambs. UCB stem cells may be a useful treatment to prevent brain injury following birth asphyxia.





O43

## Effects of antenatal magnesium sulphate treatment for neonatal neuro-protection on cerebral oxygen kinetics

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**Background:** Antenatal magnesium sulphate ($MgSO_4$) is an established neuroprotective therapy for infants of women at risk of very preterm birth. The mechanisms underlying its effects remain poorly understood. Early postnatal life is characterised by low cerebral blood flow and high oxygen consumption with elevated cerebral fractional oxygen extraction (cFTOE) preceding early neonatal brain injury. The aim of the current study was to investigate the effect of antenatal $MgSO_4$ on cerebral oxygen delivery, consumption and cFTOE in infants less than 30 weeks gestation.

**Methods:** Cerebral blood flow and tissue oxygenation index (near-infrared spectroscopy), were measured and cerebral oxygen delivery, consumption, and cFTOE calculated across the first 72 hours of life in infants ≤ 30 weeks gestation (n=64). Exposure to $MgSO_4$ for neuro-protection was defined as a 4g loading dose ± 1g per hour for a maximum of 24 hours prior to delivery.

**Results:** Thirty-six infants were exposed to $MsSO_4$. The mean (SD) maintenance dose of $MgSO_4$ received was 8g (9.5). While four infants had P/IVH at the initial NIRS study (all unilateral grade 1 IVH), fewer in the $MgSO_4$ group developed P/IVH (all grades) by day 7 of life (p=0.03). There was no difference between the groups for mean arterial blood pressure, right ventricular output, total internal carotid blood flow or cerebral oxygen delivery at any time point. Cerebral oxygen consumption and therefore cFTOE was significantly lower in those infants exposed to antenatal $MgSO_4$ (p=0.012) within 24 hours of delivery. This difference was not evident by 48 hours of age.

**Conclusions:** Preterm infants exposed to antenatal $MgSO_4$ for neuro-protection had similar systemic and cerebral haemodynamics but significantly lower cFTOE compared to infants not exposed to antenatal $MgSO_4$. These findings suggest that $MgSO_4$ balances cerebral metabolism to oxygen delivery, which may contribute to the neuroprotective actions of antenatal $MgSO_4$.

O44

## Prediction of hypothermic neuroprotection with EEG monitoring after asphyxia in preterm fetal sheep

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**Introduction:** Hypothermia improves intact recovery in term infants with moderate to severe hypoxic-ischemic encephalopathy, and is associated with reduced white and gray matter injury in asphyxiated preterm fetal sheep. It would be highly desirable to have an effective clinical biomarker of prognosis and response to hypothermia to help guide clinical use. Although recovery of EEG activity is predictive of neurological outcome after perinatal hypoxia-ischemia at term, there are only limited data on how therapeutic hypothermia affects EEG recovery at term, and none in the preterm brain.

**Methods:** Preterm (0.7 gestation) fetal sheep received (sham) umbilical cord occlusion for 25 minutes, followed by either sham hypothermia (occlusion-normothermia, n = 7), whole-body cooling from 30 minutes (occlusion-early hypothermia, n = 8) or 5 hours (occlusion-delayed hypothermia, n = 6), and continued until 72 h after occlusion.

**Results:** Early but not delayed hypothermia significantly reduced striatal neuronal loss and induction of caspase-3 positive cells after 7 days recovery, while the induction of microglia was suppressed by both early and delayed hypothermia. Greater protection with early hypothermia was associated with more rapid recovery of spectral edge frequency (by 3 h after occlusion), fewer seizures compared with occlusion-normothermia ($p<0.05$), but less suppression of EEG amplitude (0.88 ± 0.14 μV per °C, $r = 0.28$, $p < 0.001$) than delayed cooling (4.2 ± 0.26 μV per °C, $r = 0.58$, $p<0.001$).

**Discussion:** These findings suggest that in the preterm brain, greater neuroprotection with early hypothermia is associated with more rapid recovery of higher frequency EEG activity. Conversely, greater suppression of EEG activity and development of aberrant seizure activity may help identify lack of benefit during hypothermia.





O45

## Unmet oxygen demand, critical hypoxic ischaemia and brain injury in preterm newborns

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**Background:** Early intraventricular haemorrhage (IVH) in preterm infants is thought to result from inadequate oxygen delivery ($DO_2$). However the adequacy of $DO_2$ is primarily dependent on prevailing oxygen consumption ($VO_2$). We defined the concept of unmet oxygen demand as demand in excess of supply ($VO_2$ –$DO_2$) and hypothesised that unmet demand would correlate with adverse change in arterial lactate (critical hypoxic ischaemia) and IVH in preterms.

**Methods:** Cerebral blood flow (Phillips iE 33), tissue oxygenation index (Hamamatsu NIRO 200) and arterial saturation (Radiometer ABL 725) were measured and $DO_2$ (CBF • ((1.39• Hb • Hbsat/100) + (0.003 • $PaO_2$))) and VO 2 (CBF • C (a – v) $O_2$)) calculated in preterms < 30 weeks in the first 24 hours. Delta lactate (DLact) was defined as the difference between the timed and cord arterial value. Brain injury was defined as an IVH Grade II or greater by day 7.

**Results:** 83 babies with a mean (SD) gestational age of 27 (2) weeks were studied at a median (IQR) age of 14 (8, 20) hours. Fourteen had brain injury. In newborns without injury there was a weak correlation between prevailing $DO_2$ and DLact ($r = 0.337$, $p = 0.03$) but no relationship between $VO_2$ –$DO_2$ difference and DLact. In the injured group, there was a weak correlation between prevailing $DO_2$ and DLact with a significant relationship between the $VO_2$ –$DO_2$ difference and DLact ($r = 0.812$, $p = 0.008$).

**Conclusion:** Our data suggests that the oxygen delivery–consumption mismatch (unmet oxygen demand), rather than adequacy of prevailing oxygen delivery, is the likely pathophysiologic basis of brain injury in the very preterm newborn. Interventions that target unmet oxygen demand may therefore represent a novel approach to modify the risk of early brain injury in very preterm infants.

O46

## Daily high-dose caffeine administration disrupts development of Purkinje cells in the fetal ovine cerebellum

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**Background:** The standard clinical dosing of caffeine to treat apnoea of prematurity in newborn infants is often ineffective resulting in a need to administer higher doses. However, very little is known about the effects of higher doses of caffeine on the immature brain, in particular the cerebellum. Here we investigate whether repeated daily high-dose caffeine administration affects the developing cerebellum.

**Method:** High-dose caffeine (50mg/kg loading; 40mg/kg daily maintenance dose; citrate equivalent, n=9) or saline (n=8) was administered to ovine fetuses via the maternal circulation (104-118 days gestation (DG); term 147 DG). At 119 DG the cerebellum was removed and sections stained with H&E (structural analysis) or immunostained to identify microglia (Iba-1), astrocytes (GFAP), oligodendrocytes (Olig2), myelin (myelin basic protein; MBP), axons (neurofilament 200; NF200) and Purkinje cells (calbindin).

**Results:** In caffeine-exposed compared to control fetuses: (a) the area of Purkinje cell bodies was 10% smaller ($p<0.05$); (b) the area of the cerebellum, molecular layer, internal granular layer and white matter, the width of the external granular layer and the surface foliation index were similar ($p>0.05$); (c) the areal density of microglia, astrocytes and oligodendrocytes, and optical density of MBP- and NF200-immunoreactivity in the white matter were similar ($p>0.05$) and (d) GFAP-immunoreactivity in the molecular layer and internal granular layer was qualitatively similar.

**Conclusion:** Daily high-dose caffeine exposure does not affect cerebellar growth, or overtly injure the developing cerebellum in the short-term. However it may disrupt the development of Purkinje cells resulting in reduced somal growth. Further studies are necessary to confirm whether arborisation of the Purkinje cell is affected.





O47

## Is a longer better? Neuroprotection with delayed selective head cooling for three or five days after global cerebral ischaemia in the near-term fetal sheep

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Cerebral hypothermia for 72 hours reduces death and disability in neonates with hypoxic-ischaemic encephalopathy, but is only partially effective. In this study we examined whether a greater duration of head cooling could improve outcomes after global cerebral ischaemia in near-term fetal sheep.
Near-term fetal sheep (0.85 gestation) received 30 min of bilateral carotid artery occlusion followed by three days of normothermia (ischaemia-normothermia, n=8), head cooling for three days (ischaemia-three-day hypothermia, n=8) or five days of hypothermia (ischaemia-five day hypothermia, n=8) started three hours after the end of ischaemia. Sham control animals received sham ischaemia followed by normothermia (sham control, n=8). Animals were killed 7 days after ischaemia for hisopathology.
Head cooling was associated with a fall in extradural temperature to 31.3 ± 0.2°C in the ischaemia-three-day hypothermia group and 31.8 ± 0.3°C in the ischaemia-five-day hypothermia group, compared to 39.5 ± 0.1°C in the ischaemia-normothermia group ($p<0.05$). Ischaemia-normothermia was associated with a marked secondary increase in cytotoxic oedema, which was significantly attenuated in both hypothermia groups ($p<0.001$). Both hypothermia groups showed significantly better recovery of EEG power from 30 hours onwards, and higher spectral edge from 64 hours, compared to ischaemia-normothermia ($p<0.05$). Histologically, preliminary assessment suggests that the hypothermia groups showed a similar improvement in neuronal numbers. In the white matter tracts, ischaemia-normothermia was associated with a significant loss of oligodendrocytes and increase in Iba1 positive microglia. There was no significant effect of hypothermia on oligodendrocyte numbers, but Iba-1 positive microglia were markedly attenuated in both hypothermia groups ($p<0.05$).
Cerebral hypothermia for three days, started three hours after the end of ischaemia, significantly attenuated delayed cell swelling, improved recovery of brain activity and reduced inflammation but did not improve oligodendrocyte survival. There was no apparent improvement with more prolonged hypothermia for five days, suggesting that current clinical protocols are appropriate.

O48

## Chronic high-dose caffeine exposure injures the developing white matter in the ovine fetus

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**Background:** Caffeine is used extensively to treat apnoea of prematurity. As the standard dosing regimen is not always sufficient to abolish apnea, higher doses may be used. Our objective was to determine the impact of chronic, high-dose caffeine on the cerebral white matter (WM) in the very immature ovine fetus.

**Methods:** We administered a caffeine loading dose (50mg/kg; maternal i.v.) followed 2 hours later by a constant infusion (3.8mg/kg/h, maternal i.v.) to ovine fetuses (n=9) from 104 to 118 days of gestation (DG; term = 147DG); control fetuses (n=8) received saline. At 119 DG the cerebral hemispheres were immunostained to identify microglia (Iba-1), astrocytes (GFAP) and apoptosis (TUNEL assay) in the subcortical white matter (SCWM) and periventricular white matter (PVWM).

**Results:** The mean fetal plasma caffeine concentration achieved was 35±2 mg/L. There were no significant effects of caffeine on fetal arterial blood gases, glucose, lactate or pH. In caffeine-exposed animals, fetal body weight was reduced by 17% (caffeine: 2.5±0.1 vs control: 3.0±0.1 kg, p=0.014). Weights of the fetal cerebral hemispheres and the whole brain, relative to body weight, were not different between groups. In caffeine-exposed fetuses, the density of astrocytes and TUNEL positive cells was increased in the PVWM compared to control fetuses (Table 1).

| | **SCWM** | | **PVWM** | |
|---|---|---|---|---|
| | **Control** | **Caffeine** | **Control** | **Caffeine** |
| WM containing microglia (%) | 4.1±0.9 | 2.4±1.2 | 6.6±2.7 | 6.1±1.4 |
| GFAP cell density (cells/mm$^2$) | 145.9±7.9 | 145.3±9.6 | 89.1±6.2 | 122.8±8.5* |
| TUNEL cell density (cells/mm$^2$) | 1.0±0.1 | 1.2±0.1 | 1.1 ± 0.1 | 1.6±0.2* |

Table 1: Analysis of cerebral white matter (*p<0.05)

**Conclusions:** Chronic high-dose caffeine exposure reduces fetal growth, and induces cell death and astrogliosis in the PVWM. These effects cannot be attributed to fetal hypoxia and may be a direct effect of caffeine. Our study suggests that a cautionary approach is required when using chronic high-dose caffeine treatment.





# ORAL COMMUNICATIONS

Oral Session VII - Fetal & Neonatal Cardiovascular (II)

***Chairs***
***Julian T. Parer*** *(University of California - USA)*
***Jan Nijhuis*** *(Maastricht University - Netherlands)*

O49

## Does fetal growth restriction alter the cardiopulmonary and cerebral hemodynamic response to early surfactant therapy?

Malhotra Atul [1], Miller Suzanne [2], Jenkin Graham [2], Hooper Stuart [2], Allison Beth [2], Sehgal Arvind [1], Gill Andy [3], Kluckow Martin [3], Polglase Graeme [2]

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**Background:** Exogenous surfactant therapy for treatment of preterm lung disease reduces oxygen and ventilation requirements as well as the incidence of respiratory distress syndrome. However, the efficacy of surfactant therapy in growth restricted preterm neonates, who are known to have higher bronchopulmonary dysplasia rates, is unknown.

**Objective:** To compare the ventilatory, respiratory and hemodynamic response to early surfactant administration between growth restricted and appropriately grown (AG) preterm lambs.

**Design/Methods:** Ewes bearing twins underwent sterile surgery at 105 days gestation (d; term ~ 148 d) to induce fetal growth restriction (FGR) in one twin by ligation of one of its two umbilical arteries; the second twin was used as an internal control (n=4 each group). At ~123 d, fetuses were exposed and catheters and flow-probes implanted to measure pulmonary (PBF) and cerebral arterial blood flow (CBF) and arterial pressure ($P_{CA}$). Lambs were then intubated, delivered and mechanically ventilated. At 10 min, surfactant (Curosurf; 100 mg/kg) was administered intratracheally. Ventilation, oxygenation and hemodynamic responses were recorded for 1 hour before post mortem was performed. Lung tissue was collected for analysis of surfactant protein mRNA levels using qRT-PCR.

**Results:** FGR preterm lambs were 26% lighter than controls (3.03±0.48 vs. 2.25±0.41 kg; $p<0.05$) but brain weight was not different, indicating brain sparing. Endogenous SP-A, SP-B, SP-C and SP-D mRNA levels were not different between groups. Surfactant therapy reduced ventilator and oxygen requirements and improved lung mechanics, although a more rapid improvement ($P<0.05$) in lung compliance, PaO2 and increase in tidal volume was observed in AG preterm lambs. Surfactant administration to FGR preterm lambs caused a reduction in pulmonary blood flow (PBF), driven by a reduction in peak PBF ($p<0.001$) and a reduction in cerebral blood flow (CBF), driven by an increase in minimum CBF ($p<0.001$), with ductal steal (negative CBF during diastole) present in all lambs. There was little effect of surfactant administration on pulmonary or cerebral haemodynamics in AG preterm lambs.

**Conclusions:** Surfactant administration improved oxygenation and ventilation requirements similarly in growth restricted preterm lambs and AG preterm lambs. However, it resulted in reduced pulmonary and cerebral blood flow in growth restricted, but not AG, lambs indicating a different responsiveness to surfactant between FGR and AG preterm lambs.





O50

## Pravastatin rescues cardiac dysfunction during hypoxic development in the chick embryo

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**Introduction:** The beneficial effects of statins on vascular biology are being currently tested in multicentre human clinical trials to ameliorate pre-eclampsia (1). However, whether statins have beneficial or detrimental effects on fetal physiology is unknown. Here, we investigated the effects on fetal growth and cardiac function of pravastatin treatment in clinically relevant doses during hypoxic development in the chick embryo.

**Materials and methods:** Fertilised eggs were incubated under normoxic (21%) or hypoxic (14±0.5%) conditions from day 0 with pravastatin (1 $mg.kg^{-1}$) from day 13 of incubation. Statin or vehicle was injected daily into the air cell. At day 19, the embryo was killed by spinal transection. Cardiac function was assessed in the isolated heart in a Langendorff preparation. A separate cohort of animals were perfusion fixed (4% paraformaldehyde at 2.66kPa). Stereological analysis of the heart was performed on paraffin embedded sections.

**Results and discussion:** Hypoxic development promoted fetal growth restriction (Fig. 1A). Hypoxia significantly reduced the left ventricular developed pressure (LVDP, Fig. 1B) and increased the left ventricular end diastolic pressure (LVEDP, Fig. 1C), indicating impaired systolic and diastolic functions. Left ventricle wall volume was reduced (Fig. 1D), while the lumen volume was increased (Fig. 1E). Consequently, the LV wall to lumen ratio was significantly lower in the hypoxic embryos (Fig. 1F). Pravastatin had a greater protective effect on cardiac functional than on cardiac morphological changes in hypoxic embryos.

**Conclusion:** Pravastatin rescues cardiac dysfunction in the chicken embryo during hypoxic development. The data show direct beneficial effects of statins on the fetal cardiovascular system in addition to its beneficial effects on the utero-placental circulation in humans. Statins may be attractive candidate therapeutic agents against fetal origins of cardiovascular disease as well as against preeclampsia in human high risk pregnancy.

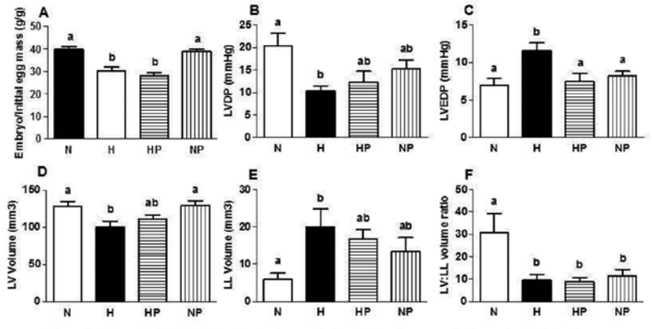


Figure 1. Means ± S.E.M. for the A, Embryo weight relative to egg weight on day 1.B, Left ventricular developped pressure. C, left venticular and diastolic pressure. D, Left ventricular wall volume. E, Left ventricular lumen volume. F, Left ventricular wall to lumen ratio. Groups are: Normoxia (N), Hypoxia (H), Hypoxia+Pravastatin (HP) and Normoxia+Pravastatin (NP). n=10, n=10, n=11 and n=10 respectively for A. n=10, n=12, n=9 and n=12 respectively fot B-F. Different letters are significantly different ($P<0.05$), One-Way ANOVA with Tukey Test.

*Supported by the British Heart Foundation*

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O51

## The effect of magnesium sulphate on the preterm fetal cardiovascular and cerebrovascular responses to profound asphyxia

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**Background:** $MgSO_4$ is commonly used for the treatment of preeclampsia and recently for perinatal neuroprotection. However, higher doses can be associated with hypotension at all ages. The effects of $MgSO_4$ on the preterm fetal cardiovascular and cerebrovascular adaptations to asphyxia are poorly understood.

**Aim:** To examine the effect of $MgSO_4$ on the cardiovascular and cerebrovascular responses to profound asphyxia in preterm fetal sheep.

**Methods:** At 104 ± 1 days (0.7) of gestation, 16 fetal sheep (n = 7 control, 7 low dose and 2 high dose $MgSO_4$) underwent 25 minutes of complete umbilical cord occlusion. Mean arterial pressure (MAP), fetal heart rate (FHR), femoral and carotid blood flows (FBF and CaBF, respectively) were measured continuously. Data were compared by two-way ANOVA with repeated measures.

**Results:** Fetal plasma Mg levels increased by approximately 15% after low-dose and 125% after high-dose $MgSO_4$ infusion. FBF and femoral vascular conductance were lower in the low dose $MgSO_4$ group during minutes 7 and 8 of occlusion compared to control ($P<0.05$). MAP, FHR and CaBF did not differ between groups during occlusion.

**Conclusions:** A 15% increase in total plasma magnesium was associated with a short-term improvement in peripheral vasoconstriction during complete umbilical cord occlusion, but was not associated with improved maintenance of MAP. Low-dose $MgSO_4$ treatment did not impair the acute cardiovascular and cerebrovascular responses to asphyxia in preterm fetal sheep. Preliminary data suggest a 125% increase in total plasma Mg does not affect the fetal cardiovascular and cerebrovascular responses to asphyxia. Further studies are required to determine the effects of clinically relevant doses of $MgSO_4$ on fetal neurological outcomes after asphyxia.





O52

## Pulmonary antioxidant capacity and oxidative stress in chronic hypoxic lambs treated with prenatal melatonin

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**Introduction:** Chronic hypoxia as in high-altitude (HA) increases reactive oxygen species (ROS) favouring oxidative stress (OS), inducing intrauterine growth restriction (IUGR) and neonatal pulmonary hypertension[1]. Melatonin is a neurohormone with important antioxidant properties. During pregnancy melatonin crosses freely the placenta and decreases OS in placenta and fetal brain[2,3]. Therefore, we hypothesise that prenatal treatment with melatonin may increase the antioxidant enzymatic capacity and reduce OS in neonatal lungs.

**Methods:** Ten HA lambs were gestated, born and studied at Putre (3,600 masl). Five received maternal oral melatonin (MM, 10mg.d$^{-1}$) and five received vehicle (CN, 5 ml.d$^{-1}$) in the last trimester of pregnancy. At 12 d old, lambs were euthanized and lung tissue was sampled. We performed protein (Western blot) and activity (colorimetric kits) assays for the AOE: superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX). Oxidative stress was measured by 8-isoprostanes and 4HNE levels (Fig. 1).

**Results and discussion:** Maternal administration of melatonin markedly decreased the antioxidant expression of SOD and CAT, and reduced the activity in all 3 enzymes in the neonatal lung. In contrast, the OS was similar among groups. We speculate that maternal melatonin may be negatively modulating ROS production in the lungs. These results diverge from melatonin effects in other fetal organs, such as cerebral and placenta[2,3] where this neurohormone enhances antioxidant enzymes.

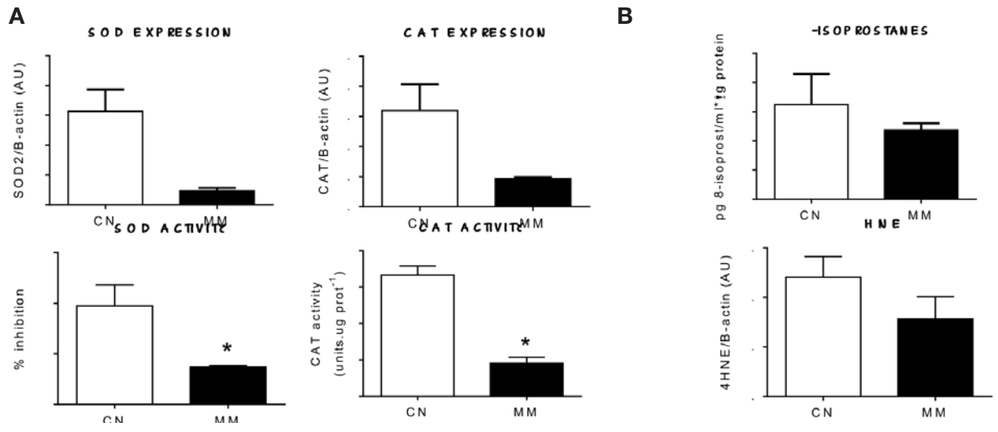


Figure 1. A. Protein expression and enzymatic activity of SOD and CAT. B. Oxidative stress markers 8-isoprostanes and 4HNE. Average ± SEM for control (CN, open bars) and prenatal melatonin treated neonates (MM, closed bars). Significant differences (t-test, $p<0.05$): * vs CN.

**Conclusion:** Prenatal melatonin diminishes the antioxidant capacity without increasing the oxidative stress in lungs of newborn lambs at HA. These differing results should be considered when proposing melatonin treatment during pregnancies affected by hypoxia.

*Supported by FONDECYT 1110595, 1120605 & 1130424.*

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O53

## Platelet counts in the first seven days of life and patent ductus arteriosus in preterm very low birth weight infants

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**Background:** Decreased platelet number and/or function is related to patent ductus arteriosus (PDA) in mice. Whether this is also the case in human infants remains controversial.

**Objectives:** To investigate the association between platelet count nadir within the first 7 days of life and the rate of hemodynamically significant PDA (HSPDA), as well as the rate of response to the treatment with cyclooxygenase (COX) inhibitors.

**Methods:** Retrospective study of a cohort of 194 very low birth weight (<1500 g) infants with gestational age <30 weeks. HSPDA was assessed by echocardiography on day of life 3.

**Results:** HSPDA was present in 105 (54.1%) infants. Of these infants 101 were treated with COX inhibitors. Treatment failure rate was 21.8%. Median platelet count nadir and rate of thrombocytopenia –defined as platelet count <150 x $10^9$/L and graded as mild (100 to <150 x $10^9$/L), moderate (50 to <100 x $10^9$/L), or severe (<50 x $10^9$/L)– within the first 2 days of life were not significantly associated with the presence of HSPDA on day 3. Moreover, low platelet counts, either on day 1-2 or day 3-7, were not significantly associated with the rate of response to treatment with COX inhibitors.

**Conclusions:** Our data provide further evidence for the lack of association between platelet counts within the first days of life and either spontaneous or pharmacological closure of the DA in VLBW infants.

O54

## Computer simulation of the cardiotocogram: the effect of catecholamines

Jongen Germaine [1], van der Hout-van der Jagt Beatrijs [2], van de Vosse Frans [1], Bovendeerd Peter [1], Oei Guid[2]

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Fetal oxygen status is an important determinant of fetal welfare. Since reliable oxygen measurements are not available yet, in current clinical practice the cardiotocogram (CTG) is used to estimate fetal welfare, via the simultaneous registration of fetal heart rate (FHR) and uterine contractions. However, these signals only show the input (uterine contractions) and output (FHR) of a complex regulation mechanism, where amongst others oxygenation plays an important role. To gain more insight into these mechanisms, a mathematical computer model was developed describing feto-maternal hemodynamics, oxygen delivery, cardiovascular regulation and uterine contractions [1,2]. Simulations of FHR responses during short term hypoxia were successful. However, if the model is used to simulate severe contraction responses, simulation results do not show FHR baseline shift or FHR overshoots as described in literature [3,4,5]. Presumably this is due to the lack of catecholamine feedback, which will lead to an increase of FHR and blood pressure [6].

Therefore we extended the model with catecholamine feedback. Catecholamines enter the blood stream via production in the adrenal medulla and spillover from nerve endings, and are eliminated in the placenta and fetal tissues [7,8]. In the model, catecholamine concentration is based on a balance between production, elimination, and convection. Due to lack of clinical data, we combined catecholamine production in the adrenal medulla and elimination in the fetal tissues into a net production, dependent on arterial oxygen concentration. Arterial catecholamine concentration was used to model an additional effect on FHR and blood pressure. Model parameters were derived from human and sheep data [3,7].

Preliminary simulation results of repeated uterine artery occlusion show an increase in FHR baseline, which are in accordance with sheep experiments [3]. Future plans include model evaluation for additional scenarios, like umbilical cord occlusion, where catecholamines are believed to play a role as well [4].

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O55

## Influence of gravity on umbilical blood flow and blood distribution during delayed umbilical cord clamping at birth

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**Background:** Delaying umbilical cord clamping (UCC) until after ventilation onset mitigates the decrease in cardiac output immediately after birth and increases placenta to infant blood transfer. It is assumed that placing the infant below the mother enhances blood transfer to the infant but no scientific information has detailed how gravity effects flow in the umbilical vessels or blood distribution between infant and placenta during delayed UCC. We hypothesize that gravity will have no net effect on flow in the umbilical vessels or blood distribution between infant and placenta.

**Methods:** At 125 days gestation lambs were delivered via caesarean section and flow probes were placed on the left pulmonary artery, carotid artery and an umbilical artery (UA) and vein (UV). Blood volumes were measured before (fetus plus placenta) and after UCC (lamb only) using biotin-labeled red blood cells. Lambs were placed 10 cm above (above; n=6) or 10 cm below (below; n=6) the mid abdomen level of the ewe and ventilation commenced. The umbilical cord was clamped 3 minutes after ventilation onset and lambs were ventilated for 30 minutes.

**Results:** Gravity had no effect on carotid artery pressure and blood flow. Compared with placing the lamb above the placenta, placing it below the placenta reduced UA flow (105±20 vs 210 ± 44 mL/min) and UV flow (84±24 vs 174±41 mL/min) following ventilation onset. As a result, pulmonary blood flow was increased (215±36 vs 137±25 mL/min) in lambs placed below the placenta, but no difference in blood volume was detected (60.6±1.6% vs 54.3±6.0% of fetus plus placenta).

**Conclusion:** Following ventilation onset, vertical position above or below the placenta did not affect the net transfer of blood between the lamb and placenta during delayed UCC. In lambs placed below the placenta, flow in the UA and UV were decreased equally.

O56

## Repeated acute on chronic lipopolysaccharide exposure is associated with suppression of fetal heart rate variability in preterm fetal sheep

Lear Christopher A [1], Davidson Joanne O [1], Booth Lindsea C [1], Wassink Guido [1], Galinsky Robert [1], Drury Paul P [1], Fraser Mhoyra [1], Bennet Laura [1], Gunn Alistair J [1]

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Perinatal exposure to infection is highly associated with adverse outcomes. Experimentally, acute, severe exposure to gram negative bacterial lipopolysaccharide (LPS) is associated with increased fetal heart rate variability (FHRV). It is unknown whether FHRV is affected by subclinical infection with or without acute exacerbations. We therefore tested the hypothesis that FHRV would be associated with hypotension after acute on chronic exposure to LPS. Chronically instrumented fetal sheep at 0.7 gestation were exposed to a continuous low-dose LPS infusion (n = 12, 100 ng over 24 hours, followed by 250 ng/24h for a further 96 hours) or the same volume of saline (n = 10). Boluses of either 1 μg LPS or saline were given at 48, 72 and 96 hours. Low-dose infusion was not associated with hemodynamic or FHRV changes. LPS boluses were associated with tachycardia and suppression of nuchal electromyographic activity in all fetuses. 7/12 fetuses developed hypotension (a fall in mean arterial blood pressure ≥ 5 mmHg). FHRV was transiently increased only at the onset of hypotension, in association with increased cytokine induction and EEG suppression. FHRV then fell before the nadir of hypotension, with transient suppression on one measure of short-term variation. After the second LPS bolus, the hypotension group showed a biphasic pattern of a transient increase in FHRV followed by more prolonged suppression. These findings suggest that it is the onset of infection-related hypotension in the preterm fetus, rather than exposure to high-dose LPS itself, that is associated with transiently increased FHRV, and that repeated exposure to LPS leads to progressive loss of FHRV.





# ORAL COMMUNICATIONS

Oral Session VIII - Nutrition and growth & Translational studies

***Chairs***

***Tim Moss*** *(Monash University, Australia)*

***Jan Derks*** *(University Medical Center - Utrecht, Netherlands)*

O57

## Cytomegalovirus during pregnancy: outcomes of non primary versus primary maternal infections

Simonazzi Giuliana [1], Cervi Francesca [1], Puccetti Chiara [1], Daveri Valentina [1], Contoli Margherita [1], Curti Alessandra [1], Lazzarotto Tiziana [2], Rizzo Nicola [1], Guerra Brunella [1]

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**Background:** Cytomegalovirus (CMV) is the most common cause of intrauterine infection. Unlike other infections, preconceptional immunity against CMV provides only a partial protection and intrauterine transmission of CMV has been reported in 0.5% to 2.2% of cases. Although, it is generally accepted that symptomatic congenital infections are rare, in recent years there is an increasing evidence that-non primary maternal infection may be a significant cause of severe congenital disease.

**Objective:** To evaluate the pregnancy outcomes (maternal-fetal rate of transmission and incidence of symptomatic congenital disease) of non-primary maternal infections versus primary infections.

**Study design:** Retrospective study of pregnant women with active CMV infection, referred to our units in a 13 years period (1999-2011). Patients were divided into 2 groups according to the results of confirmatory serologic testing (avidity test, immunoblotting): serologic profile consistent with non-primary infection and serologic profile suggestive of primary infection. We compared the vertical transmission rate, the sensibility and specificity of amniocentesis and the percentage of symptomatic infections into the two groups.

**Results:** Long term follow-up was available in 1099 cases: 188 non-primary infections and 911 primary maternal infections. Congenital infections were diagnosed in 9 (4.79%) fetuses/neonates of non-primary infection group and in 218 (23.92%) fetuses/neonates of primary infection group (p<.001). 553 amniocentesis were performed in primary infection group with a sensibility of 70.5% and specificity of 99.5%. In non-primary infection group 14 amniocentesis were performed, with a 100% of sensibility and specificity. Numbers of symptomatic infected fetuses/neonates were not statistically different into the two groups: 2 (22.2%) in non-primary infections versus 48 (22.0%) in primary infections (p= n.s.).

**Conclusion:** Although pre-existing maternal immunity significantly reduces maternal-fetal transmission, the rate of symptomatic congenital CMV disease is similar in both infection groups. Amniocentesis could be useful to identify infected and non infected fetuses/newborns in both cases.





O58

## Amnion cells activate endogenous lung progenitors to augment lung repair

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[3] Harvard Stem Cell Institute, Harvard University, Boston, USA

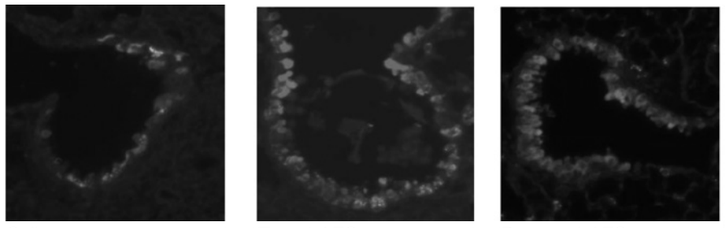
**Background:** Amnion epithelial cells (hAECs) are known for their protective and reparative properties in the lung. Recent investigations indicate that preterm hAECs are less able to mitigate lung repair compared to term hAECs. This suggests that hAECsmmay differ in their regenerative and reparative potential depending on the gestational age when procured.

**Aim:** Determine differences between hAECs from term versus preterm amnions in their ability to activate bronchioalveolar stem cells (BASCs).

**Methods:** 0.4 IU bleomycin was administered intranasally to 6-week old C57Bl6 mice. After 24 hours, a single 200uL intraperitoneal injection of hAECs (4 million, term or preterm) or saline was administered to each mouse. Assessment of BASC recruitment and function was determined using immunohistochemistry (CCSP/SPC) and a combination of flow cytometric sorting and 3D co-culture, respectively.

**Results:** Term hAECs triggered an early activation of the stem cell niche at the bronchioalveolar junction. This was not observed the preterm hAEC group. At day 7, average BASC numbers were ~2-fold higher in term hAEC treated animals compared to controls (*p<0.05), while BASC numbers were significantly lower in preterm hAEC treated animals (*p<0.05). By day 14, the bronchioalveolar stem cell niche returned to quiescence in term hAEC treated animals but remained active in control and preterm hAEC treated animals. When BASCs were co-cultured, term hAECs encouraged development of larger alveolar colonies compared to preterm hAECs.

**Conclusion:** Resolution of bleomycin induced lung injury mediated by term hAECs corresponds to a transient increased activation of the bronchioalveolar junction niche. Term hAECs also appear to support BASC growth *in vitro.* Prematurity impacts the ability of hAECs to activate this stem cell niche and may explain the reduced ability for preterm hAECs to mitigate lung injury. These findings have profound implications on autologous banking of amnion cells and other stem cell types.



*Saline* *Term hAECs* *Preterm hAECs*

Immunohistochemical staining for BASCs at terminal bronchioles (CCSP/SPC) 7 days after bleomycin administration.

O59

## Reduced uterine blood flow induces asymmetric growth restriction in spiny mouse fetuses

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The Ritchie Centre, MIMR-PHI Institute, Monash University, Melbourne, Australia

**Introduction:** Intrauterine growth restriction (IUGR) is a major complication of pregnancy leading to significant perinatal morbidities and mortality. Typically, induction of IUGR in animal models involves profound reduction of uterine or placental blood flow by occlusion/ablation of blood vessels, leading to reduced fetal growth. Here we show, in the spiny mouse, that IUGR can occur simply by restricting the progressive increase in uterine artery size from 0.7 gestation.

**Methodology:** At 27 days gestation (term is 38-39 days), using aseptic techniques under general anaesthesia, we placed a 4 mm length of silastic tubing (0.8mm I.D) around the left uterine artery, superior to the ovarian artery (n=4). Sham controls (n=3) underwent surgery without cuff placement. The dam was recovered and carried her pregnancy to 37 days gestational age when she was killed and all fetuses collected. Biometry measures were taken and tissues were weighed and collected for subsequent analysis.

**Results:** All dams carried their pregnancies to 37 days gestation without fetal loss. Fetuses in the left horn were always smaller (20-30%) than controls. The placenta and brain to body weight (BW) ratios were significantly increased, and kidney, liver and GIT weights relative to BW were decreased in IUGR offspring compared to controls. Lung and carcass relative to BW were not different between groups.

**Discussion:** In the spiny mouse, asymmetric fetal growth restriction, without fetal loss occurs when the increase in uterine artery diameter is prevented during late gestation. This model of IUGR appears to closely mimic human pregnancies with IUGR where late gestation fetal loss is rare. We are now characterising the pathological implications in a range of organs, subsequent to reduced utero-placental blood supply and IUGR. We will then use this model to test therapeutic strategies aimed to improve outcomes for the growth-restricted fetus.





O60

## The effects of obesity on hippocampal function in the rat

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**Introduction:** Obesity is a low-grade inflammatory state, which constitutes an inhospitable intrauterine environment. After birth, children of obese mothers are further at increased risk of neurodevelopmental abnormalities, eg reduced cognitive capacity, developmental delay, attention deficit hyperactivity disorder, and autism spectrum disorders. In adults, high BMI and metabolic syndrome are associated with lower cognitive performance, and poor executive function and memory. The hippocampus is a major centre for memory, learning and decision making, and is important in emotional regulation. The aim of the present study was to determine the effects of obesity *in utero* and beyond, on hippocampal function in rat.

**Methods:** Dams were placed on control chow or a high fat (HF) diet for 6 weeks and then mated with lean males. Offspring were retained on the diet of their dam. At 7 weeks of age, cognitive function was assessed using behavioural testing. Subsequently hippocampal brain slices were studied electrophysiologically using multielectrode arrays.

**Results:** The Barne's maze showed enhanced learning in males on HF diet but not in females, while memory was strikingly poor in HF fed females. With the Elevated Plus maze, HF males spent less time in the open arms suggesting anxiety-like behaviour. In hippocampal slices, HF diet had a greater effect on long-term potentiation (LTP) in HF males vs control fed. A significant observation was that in all our obese males, slices of hippocampus had a dramatically greater tendency to produce oscillatory network (epileptiform) activity compared with lean counterparts.

**Conclusions:** Obesity affects behaviour and hippocampal function in a sex-dependent manner. These effects include epileptiform/hyperactivity that occurs in obese males. Since hippocampal hyperactivity has been strongly implicated as underlying at least some of the age-related cognitive impairment, obesity may exacerbate age-related cognitive impairment, either in terms of earlier onset (premature aging) and/or degree of severity of impairment.

O61

## Maintenance of human amnion epithelial cell phenotype in pulmonary surfactant: a potential novel combination therapy

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**Introduction:** Preterm newborns often require mechanical respiratory support that can result in ventilation-induced lung and brain injury despite exogenous surfactant treatment. Human amnion epithelial cells (hAECs) can reduce lung and brain inflammation; and administration in surfactant is a potential therapy. We aimed to determine whether hAECs remain viable and maintain function after combination with surfactant *in vitro*, and if cells delivered in surfactant can reach the brain after 2 h of mechanical ventilation *in vivo*.

**Methods:** *In vitro* studies: hAECs were incubated in surfactant (Curosurf) or PBS for 30 min at 37°C. Cell viability, phenotype (by flow cytometry), inhibition of T-cell proliferative responses and differentiation into lung epithelial-like cells was investigated. *In vivo* studies: Lambs were delivered at 126 days of gestation (term is 147 days). $90x10^6$ hAECs were labeled with a fluorescent iron label and administered intratracheally in 3ml surfactant, before the initiation of ventilation. Lambs were injuriously ventilated (tidal volume targeting 15 mL/kg, no positive end-expiratory pressure) for 15 min and then gently ventilated for 105 min. MRI was performed at 120 min. Brains were collected and analysed for histopathology and cell tracking.

**Results:** Cell viability and apoptosis of hAECs were not altered after combination with surfactant, and hAEC phenotype was not altered. Immunosuppression of T-cells by hAECs was not altered after combination with surfactant. hAEC differentiation into lung epithelial-like cells was equivalent after exposure to PBS or surfactant, and SP-A expression was equivalent. Following injurious ventilation, hAECs delivered intratracheally in surfactant were found in the brain.

**Conclusion:** Surfactant exposure does not alter viability or function of hAECs. A therapy consisting of hAECs administration in surfactant may be efficacious to ameliorate or prevent preterm lung and brain injury.





O62

## A new 3 tiered approach predicts risk for major pregnancy complications

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The major complications of late pregnancy, preterm birth (PTB), preeclampsia (PE), intrauterine growth restriction (IUGR) and gestational diabetes mellitus (GDM), together afflict about 25% of first pregnancies and rising. Furthermore, women who suffer one of these complications, and their offspring, are at greater risk of adult onset chronic disease than those who do not. Currently there are no reliable methods to identify women at risk in first pregnancies. We aimed to develop algorithms combining clinical, lifestyle and SNP genotype data for use as screening tools in early pregnancy to identify risk for PTB, PE, IUGR and GDM.

Data from 2977 women and their partners who participated in the SCOPE study in Adelaide and Auckland were included. Blood was sampled for DNA extraction and SNP genotyping. Clinical and lifestyle information and pregnancy outcomes were recorded for all women, including 123 PTB, 167 PE, 142 SGA and 89 GDM pregnancies. Two prediction models for each pregnancy complication were developed by penalized logistic regression, correction for FDR with final integration using Baye's theorem. Risk stratification was obtained in 3 tiers: low, moderate and high risk for each complication.

Models included clinical, lifestyle and SNP genotypes for each complication with SNPs contributing up to 53% of weighted risk prediction. The percentage of women predicted at low risk who subsequently developed the complication, i.e. false negatives, was 1.3% PTB, 1.5% PE, 2.1% IUGR and 0.7% GDM. Positive predictive values in women deemed at high risk were 23.6% for PTB, 22.9% for PE, 20.4% for IUGR and 21.8% for GDM. Our models accurately predicted 88% of PTB, 91% of PE, 86.6% of IUGR and 92% of GDM cases.

We now need to validate our models prospectively in new cohorts. These screening tools, if confirmed, may enable early identification of women at risk in the clinic.

O63

## Lung aeration at birth in a rabbit model of congenital diaphragmatic hernia

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Congenital diaphragmatic hernia (DH) occurs in ~1:4000 infants. DH allows abdominal contents to enter the chest causing fetal lung hypoplasia. This results in severe respiratory insufficiency at birth. Currently there is little guidance how to best resuscitate CDH infants at birth. As DH is usually one-sided, the ipsilateral lung is mechanically different to the contralateral lung. We aimed to assess the rate and degree of lung aeration at birth between the left and right lungs in DH rabbits using synchrotron-based phase contrast X-ray (PCX) imaging.

Pregnant rabbits underwent surgery at 25 days gestation (dG; term31-32dG) to induce left-sided DH in two fetuses per litter. At 30dG kittens were intubated, delivered and ventilated. Peak inflation pressure was adjusted to attain 5mL/kg tidal volume. A 5cm$H_2O$ end expiratory pressure was maintained throughout. PCX imaging (24keV; 5Hz at 20msec/frame at the Spring8 synchrotron in Japan) commenced prior to ventilation onset.

Fifty-eight percent of DH fetuses survived. The body weight of DH kittens was similar to un-operated littermate controls but there was a 25% reduction in lung-to-body weight ratio in DH (0.054 ± 0.003; n=6) compared to control (0.072 ± 0.003; n=8; p< 0.0005) kittens. In DH kittens the right lung aerated ~60% more rapidly than the left (Control 0.034 ± 0.007 mL/sec vs DH 0.014 ± 0.004 mL/sec; p<0.005). By 30 seconds, 67 ± 4% of the air was in the right lung and only 33 ± 4% of the air was in the left lung (p<0.005).

The contralateral right lung aerates more rapidly than the ipsilateral (DH side) left lung, increasing the risk of over-distension injury in the right lung and atelectasis injury in the left lung. This imaging technique will allow us to discern which resuscitation techniques most effectively aerate the lung and reduce lung injury in CDH infants at birth.





O64

## Prenatal melatonin extends gestation and decreases fetal growth in high-altitude sheep

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**Introduction:** High altitude (HA) neonates present intrauterine growth restriction (IUGR)[1].Oxidative stress is enhanced in chronic hypoxia[2]. This condition may program cardiovascular dysfunction later in life[3]. Melatonin is a potent endogenous antioxidant that neither the fetus nor the early neonate are able to synthesize[4]. In this study, we determined the effects of prenatal melatonin (given to the pregnant ewe) in the newborn lamb gestated under chronic hypoxia of altitude. We evaluated biometric variables, plasmatic melatonin and oxidative stress markers.

**Methods:** Twelve ewes were bred under chronic hypobaric hypoxia and gave birth to 12 newborn lambs (Putre, 3.600masl). Six ewes conform a control group (CN, 5 ml vehicle) and 6 the melatonin treated group (MM, 10 mg.$d^{-1}$ melatonin in 5 ml ethanol 1.4%). Treatments were given to the pregnant ewes, daily at 18h (oral), in the last third of gestation (100-150 d). We determined gestational length, weight and neonatal biometry in the first 10 days of life. Maternal and neonatal plasma samples were obtained to determine antioxidant capacity (FRAP, plasma reducing capacity) and melatonin levels (RIA). Finally, plasmatic 8-isoprostanes concentration was used as markers of oxidative stress.

**Results and discussion:** Prenatal melatonin treatment extends gestation by 3% (5 days), shifts birth time and reduces birth weight (Table 1). Furthermore, plasmatic melatonin increases in both groups during night, but it was doubled in treated ewes relative to controls (Table 1). Melatonin treatment increased FRAP in both, ewes and neonates. Further, melatonin induces decreases in plasmatic 8-isoprostanes in ewes. However, melatonin increases plasmatic 8-isoprostanes in neonates (all differences p<0.05).
**Conclusion:** Maternal melatonin impairs fetal growth and increases oxidative stress in neonates. Future studies are needed to clarify the mechanisms involved in these findings and the potential prolonged effects in adult life. Caution should be taken when considering antenatal treatment with melatonin in chronic hypoxic pregnancies.

| | CN | MM |
|---|---|---|
| Gestational length (days) | 150 ± 2 | 155 ± 4* |
| Time of birth (hour) | 06 - 12 | 16 - 24* |
| Birth weight (kg) | 3.45 ± 0.45 | 2.88 ± 0.54* |
| Plasma melatonin AM (pg/ml) | 248 ± 164 | 176 ± 107 |
| Plasma melatonin PM (pg/ml) | 600 ± 386 | 1400 ± 476* |

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# POSTER PRESENTATION

P1

## Type 1 cannabinoid receptors blockade simultaneous to nociceptive stress during lactation, perturb the hepatic endocannabinoid system leading to lipid accumulation in liver of adult mice

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**Background:** Type 1 cannabinoid receptors ($CB_1R$), anandamide, 2-arachidonoyl-glycerol, and the enzymes fatty acid amide hydrolase (FAAH) and monoacylglicerol-lipase (MAGL) constitute part of the endocannabinoid system (ECS). $CB_1R$ participates in the hypothalamic feed-back mechanism modulating the stress response, which is exacerbated by blocking it with its antagonist AM-251. We have previously shown that nociceptive stress (NS) simultaneous to $CB_1R$ blockade during early lactation, cause insulin resistance (IR) in adulthood.

**Aim:** Since hepatic $CB_1R$ is required for diet-induced IR; we evaluated the hepatic ECS in adult mice subjected to manipulations during lactation.

**Methods:** From day 1 to 10 of lactation, pups were daily treated with an oral dose of AM-251 and 1 h later subjected to NS with a subcutaneous injection of saline in the back. This was the main AMNS group. Appropriate controls were also designed: NS and vehicle-treated (NSV), AM-251-treated no NS (CAM) and vehicle-treated no NS (CV). At 140 days, mice were euthanized and the liver extracted. Hepatic free fatty acids (FFA) and triglycerides (TG) were measured, and expression of $CB_1R$, FAAH, and MAGL evaluated by RT-PCR and Western Blots. Activity of FAAH and MAGL were also measured

**Results:** mRNA expression was not different in any group. However, protein levels of $CB_1R$ in AMNS group were significantly higher in comparison to CV. Although FAAH and MAGL proteins were not different in AMNS group compared to CV, FAAH activity in AMNS was significantly lower, while MAGL did not change. Hepatic TG concentration was significantly increased by 2 fold in AMNS group.

**Conclusions:** $CB_1R$ blockade together with NS during early lactation, reprogramme the hepatic SEC in adult mice by elevating the amounts of $CB_1R$ protein and decreasing FAAH activity. This concerted mechanism, should result in sustained overactivity of $CB_1R$, with long term consequences in accumulation hepatic triglycerides and associated metabolic alterations.

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P2

## Maternal body mass index influences umbilical artery doppler velocimetry in physiologic pregnancies

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**Objectives:** To assess if there is a relationship between maternal Body Mass Index and Umbilical Artery Doppler velocimetry in physiologic pregnancies.

**Methods:** Healthy pregnancy women, referred to our center at or before 32 weeks of gestation, were recruited. According to Body Mass Index, they were divided into underweight, normal weight, overweight and obese women. At 32+0 weeks of gestation, maternal Body Mass Index and Umbilical Artery Doppler velocimetry was recorded. A correlation between Umbilical Artery Pulsatility Index and Body Mass Index was assessed by ANOVA Test, t-Sudent's test and linear regression.

**Results:** 185 women were included. Mean Pulsatility Index of Umbilical Artery at 32+0 was significantly higher in obese women (0,95±0,01 vs 0,87±0,01 vs 0,67±0,01 vs 0,47±0,03; $p<0,05$). We found a positive correlation between PI-UA and maternal BMI ($r2=0,6$; $p<0,05$)

**Conclusions:** There is a positive correlation between body mass index and Pulsatility Index of Umbilical Artery. If our data will be confirmed, maternal body mass index should be considered in evaluation of Umbilical Doppler velocimetry.

P3

## Connexin hemichannel blockade after fetal cerebral hypoxia-ischaemia improves survival of striatal phenotypic neurons

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**Background:** Basal ganglia damage after asphyxia at term remains common and is closely associated with later cerebral palsy. After asphyxia, connexin hemichannels can open, leading to , calcium influx and release of ATP and glutamate resulting in neuronal death.

**Aim:** To examine the hypothesis that connexin hemichannel blockade after global cerebral ischaemia improves survival of striatal phenotypic neurons.

**Methods:** A mimetic peptide that blocks connexin 43 hemichannels was infused into the lateral ventricle of chronically instrumental fetal sheep in utero at 128 ± 1 days (0.87) of gestation. Short (1 h, n = 5) or long (25 h, n = 6) infusion of peptide or vehicle (n = 6) was started 90 minutes after 30 minutes of severe ischemia induced by reversible bilateral carotid artery occlusion. Sheep were killed 7 days later and fetal brains were processed for neuropathological assessment.

**Results:** Cerebral ischemia was associated with reduced numbers of calbindin-28k, GAD and ChAT-positive neurons ($P<0.05$ vs. sham occlusion) but not nNOS-positive neurons. Long infusion of peptide was associated with increased survival of calbindin 28k-postive striatal neurons ($P<0.05$ vs. saline + occlusion) but did not affect survival of GAD, ChAT or nNOS-positive neurons. The short infusion of peptide did not improve survival of striatal neurons compared to the saline + occlusion group.

**Conclusions:** These data suggest that connexin hemichannel blockade after asphyxia improves survival of striatal phenotypic neurons and has the potential to help reduce basal ganglia injury in asphyxiated infants.





P4

## Spontaneous pre-existing hypoxia does not affect brain damage after global cerebral ischaemia in the near-term fetal sheep

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There is increasing evidence that a mild insult before a subsequent severe, injurious insult may protect (pre-condition) the brain from injury, although the effects are highly time dependent. The aim of this study was to determine whether spontaneous mild, pre-existing hypoxia would reduce injury from a later period of 30 min of global cerebral ischaemia in the near-term fetal sheep.

Chronically instrumented fetal sheep at 0 .85 gestation were assigned based on their baseline $PO_2$ measurements to the normoxia ($PO_2$ > 17 mmHg, mean±SEM 16.1 ± 0.6 mmHg, n =7) or pre-existing hypoxia ($PO_2$ < 17 mmHg, 26.0 ± 1.1 mmHg, n = 9) group. Fetuses received 30 min of global cerebral ischaemia induced by bilateral carotid artery occlusion or sham ischaemia (n = 7) and were allowed to recover for seven days.

Ischaemia was associated with secondary cell swelling and seizures, reduced final EEG power, loss of sleep state cycling and significant loss of neurons and oligodendrocytes ($p<0.05$). Pre-existing hypoxia was associated with a significantly attenuated rise in fetal mean arterial pressure from 18 to 36 hours after ischaemia and slower resolution of cortical cytotoxic oedema from 96 to 150 hours. However, pre-existing hypoxia did not affect recovery of EEG power, sleep state cycling or neuronal and oligodendrocyte survival compared to normoxic animals.

These data suggest that spontaneous mild pre-existing hypoxia is not associated with preconditioning of the brain against injury from global cerebral ischaemia in the near-term fetal sheep. We speculate that long-standing hypoxia may be associated with resolution of the neuroprotective phenotype that is reported after acute hypoxic exposure.

P5

## Neurotrophin levels across gestation and in infants born small for gestational age

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**Background:** While those infants born very preterm (i.e. less than 32 weeks gestation) experience the most severe neurodevelopmental outcomes, infants born during the late preterm period (33-36 weeks gestation) still demonstrate subtle neurodevelopmental deficits. Further, infants born small for gestational age (SGA, <10$^{th}$ birth weight centile) exhibit poor cognitive and motor function compared to infants born an appropriate size for gestation. Neurotrophic factors are proteins involved in neural growth, survival and differentiation, and are critically important for in-utero development of the central nervous system. The aim of this study was to assess levels of brain derived neurotrophic factor (BDNF), neurotrophic factor-3 (NT3) and neurotrophic factor-4 (NT4) across gestation and in infants born small for gestational age. Further, maternal and obstetric factors that have either been linked to poor developmental outcomes and/or known to affect fetal growth were investigated.

**Methods:** Cord blood from 145 preterm infants (≤32 weeks), 139 late preterm infants (33-37 weeks) and 118 term infants (38-41 weeks) was collected at delivery at the Women's and Children's Hospital, Adelaide, and BDNF, NT3 and NT4 levels were measured by ELISA. Customised birth weight centiles were calculated using the online calculator (www.gestation.net). Maternal and obstetric data was recorded from hospital records.

**Results:** Cord blood NT3 and NT4 increased across gestation ($p<0.001$), while BDNF levels remained stable. BDNF, NT3 and NT4 were not affected by size at birth, with levels in SGA infants equal to those in appropriately grown and large for gestational age infants. Neurotrophin levels did not change with maternal smoking, BMI and gestational diabetes. Early onset pre-eclampsia was, however, associated with decreased BDNF in preterm infants.

**Conclusions:** NT3 and NT4 levels increase across gestation, but do not change with infant size at birth. BDNF levels remain unchanged across gestation, but are decreased in preterm infants born to mothers with pre-eclampsia. These data warrant further investigation, given that decreased neurotrophin levels have been associated with long-term neurodevelopmental outcomes, including cognitive deficits, schizophrenia and autism.





P6

## Progesterone replacement therapy following preterm birth increases circulating cortisol in male neonates

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**Background:** Preterm birth results in the early separation of the infant from the placenta and hence its large supply of progesterone and the neuroactive metabolite allopregnanolone. Allopregnanolone promotes brain development such as myelination and has neuroprotective properties. The effect of postnatal progesterone replacement was assessed in promoting myelination in the preterm neonatal brain.

**Method:** Guinea pig neonates were delivered preterm (GA62) and term (GA69) and maintained until term equivalency (PND 8 and 1 respectively). The preterm neonates received progesterone (16mg/kg) or vehicle subcutaneously daily. Expression of myelin basic protein (MBP) and myelin proteolipid protein (PLP) were quantified by immunohistochemistry and PDGFra and OLIG2 protein were assessed by western blot in cerebellar samples. Plasma allopregnanolone and cortisol concentrations were measured by radioimmunossay and ELISA respectively.

**Results:** Progesterone replacement did not increase circulating allopregnanolone concentrations in male neonates however did increase circulating cortisol concentrations ($p<0.05$). Term equivalent male neonates born preterm demonstrated significantly lower PDGFRa, OLIG2, MBP and PLP expression in the cerebellum ($p<0.05$), which was exacerbated following progesterone treatment. Female preterm neonates exhibited similar circulating allopregnanolone and cortisol concentrations and oligodendrocyte expression in the cerebellum compared to term.

**Conclusions:** Progesterone metabolism markedly differs in male and female preterm neonates with male neonates exhibiting raised cortisol concentrations. Male preterm neonates remained developmentally immature and increased cortisol, previously shown to reduce myelination, may further increase this deficit. Further investigation of the use of progesterone therapy during pregnancy and in early neonatal life with consideration to the sex of the offspring is warranted.

P7

## Neuroprotective effects of melatonin in hypoxic-ischemic brain injury of preterm infants

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White matter (WM) injury is a common problem in preterm infants, and can lead to severe complications such as cerebral palsy. It is known that oxidative stress and inflammation mediate preterm brain injury. Melatonin (MLT) is a hormone that has potent antioxidant, anti-apoptotic and anti-inflammatory capacities thus can potentially be used to prevent brain injury. The aim of this study was to investigate the neuroprotective properties of low-dose MLT administered 2h *after* umbilical cord occlusion (UCO) in preterm fetal sheep.

Fifteen fetal sheep underwent surgery at 98 days of gestation, and were divided into three groups, UCO, UCO+MLT, and control (sham-UCO). At 103 days of gestation, UCO was performed for 25 min, or until the fetal mean arterial pressure (MAP) dropped to <10 mmHg. The UCO+MLT group received low-dose MLT (0.2mg bolus followed by 0.1mg/h infusion for 24h i.v. directly to ewe) commencing at 2 h post-UCO. Fetal hemodynamics and EEG background were monitored throughout the experiment. Post-mortem occurred 10 d post-UCO, animals were then euthanized, and fetal brains collected for histolopathology.

UCO caused acute severe hypoxia and lactic acidosis that was recovered by 4 h post-UCO, and these conditions were not affected by MLT administration. Fetal brain EEG amplitude and spectral edge frequency were suppressed after UCO, and the suppression was less profound with MLT treatment. Delayed-onset seizures were observed in the UCO group, and their total number and duration were lower following MLT administration. UCO caused an increase in the number of activated microglia and caspase-3 positive cells, and a decrease in myelinating axons. MLT treatment was able to reduce apoptosis and preserve myelination.

UCO altered brain activity, increased the number of seizures, and significantly increased the number of inflammatory and apoptotic cells. MLT treatment ameliorated these effects. Low-dose Maternal MLT treatment may be an ideal therapy for WM injury in preterm infants.





P8

## Antenatal azithromycin treatment for intra-amniotic ureaplasma infection improves neurobehavioral and cognitive development in prematurely born rhesus macaques

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**Objective:** Intra-amniotic infection (IAI) by *Ureaplasma* is a major cause of preterm birth and is associated with respiratory distress (RDS) and neurodevelopment disabilities. We have shown that treatment of *U.parvum* infection with azithromycin (AZI) reduces the severity of fetal lung injury and inflammation in the non-human primate (NHP). Long-term neonatal outcomes following antenatal antibiotics have not been assessed. Our objective is to examine the neurobehavioral and cognitive development of prematurely born NHP exposed to chronic *U.parvum* and antibiotic therapy *in utero*.

**Methods:** Pregnant rhesus macaques were assigned to control (CON; n=2), *U.parvum* alone (IAI; n=3) or IAI *plus* AZI treatment (AZI, 25mg/kg/d; IV, for 7d; n=2) groups. Animals were inoculated with *U.parvum* ($10^5$cfu) at 120 dGA. Treatment was initiated 20 days after inoculation. Infants were delivered between 141-152 dGA (term ~168d). Infants received continuous 24-hr intensive care, including respiratory support, IV fluids and nutrition. APGAR scores were recorded (5, 20mins). Growth and morphometric data were collected, cognitive testing and Primate Neonatal Neurobehavioral Assessments (PNNA) were performed over 6-months.

**Results:** APGAR scores were lower in infected infants compared to control and AZI animals (3.0±1.0 *vs.* 5.0±1.2 *vs.* 7.0±0.8, respectively). Infants exposed to *U.parvum* had mild-to-moderate RDS and were supported with supplemental $O_2$ and/or assisted ventilation (PPV, CPAP). Mechanical ventilation & more nutritional support was required for neonates exposed to *U.parvum* IAI alone compared to controls and AZI animals. Cognitive, sensorimotor and attention deficits were observed more in infants exposed to *U.parvum* IAI alone compared to controls and AZI animals.

**Discussion:** Preliminary observations suggest reduced levels of clinical care and improved cognitive and learning outcomes for AZI treated infants compared to untreated infants. This work provides very compelling initial evidence for the beneficial effects of antenatal antibiotic therapy for the treatment of *Ureaplasma* IAI and for improved perinatal health outcomes.

P9

## Pre- and post- natal effects of melatonin on pulmonary prostanoids function in chronic hypoxic lambs

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**Introduction:** Neonatal pulmonary arterial hypertension (NPH) courses with increased contractile pathways, creating an imbalance in cardiovascular homeostasis. Prostanoids are key molecules for this balance, such as the vasodilator prostacyclin (PGI2) and the vasoconstrictor thromboxane (TX)[1]. Gestation at high altitude (HA) induces NPH[2] and melatonin has been proposed as a modulator of vascular function in the perinatal period[3,4]. In this study, we analyzed the effects of perinatal melatonin on neonatal pulmonary protein expression of prostanoid enzymes cyclooxygenases (COX1, COX2), thromboxane synthase (TXs) and prostacyclin synthase (PGI2s).

**Methods:** 17 sheep were gestated under chronic hypoxia (Putre, 3.600masl). At midgestation, they were randomly divided in a control group (CN, 5 mg.d$^{-1}$ of vehicle, n=6), prenatal melatonin (MM, 10 mg.d$^{-1}$ in the last third of gestation, n=5 ) and postnatal melatonin (MN, 1 mg.kg$^{-1}$.d$^{-1}$, 3-12 postnatal days, n=6). All treatments were administered daily at 18h (oral). Lambs were euthanized at 12 days old and lung tissue was collected. Protein expression (Western blot) was quantified for COX1, COX2, PGI2s, and TXs. Results were expressed as mean ± SEM and analyzed with Student t-test ($p<0.05$).

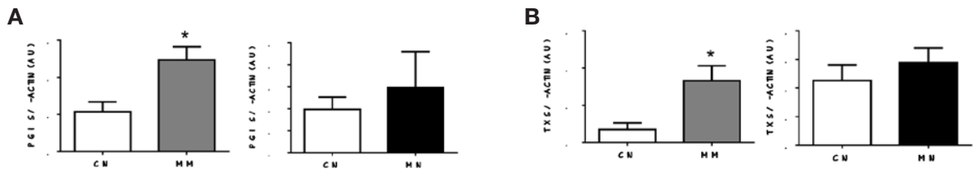


Figure 1. Pulmonary protein expression of PGI2s (A) and TXs (B). Significant differences ($p <0.05$): * vs CN.

**Results and discussion:** Both melatonin treatment, MM and MN, show a significant increase in COX 1, COX2 and TXs pulmonary expression ($p <0.05$). However, pulmonary PGI2s expression was similar in the 3 groups.

**Conclusion:** Our study shows that melatonin modulates an increased function of prostanoids, apparently towards a vasoconstrictor function in the chronic hypoxic neonatal lung. Further experiments should focus on the involved mechanisms of these effects.

*Supported by FONDECYT 1110595, 1120605 & 1130424.*

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P10

## PDGF expression and pulmonary artery remodeling in newborn lambs differentially exposed to perinatal hypoxia

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**Introduction:** Perinatal chronic hypoxia result in pulmonary artery hypertension and remodeling, This maladaptive response does not revert in all the cases when normoxic conditions are restablished (1, 2). PDGF pathways are potentially involved in such remodeling (3). Here, we compared the pulmonary PDGF expression and artery remodeling in four groups of lambs differentially exposed to perinatal hypoxia.

**Materials and methods:** Six high altitude newborn lambs fully gestated and studied at highlands (HHH), six low altitude newborn lambs fully gestated and studied at lowlands (LLL), five newborn lambs partially gestated at highlands but returned to lowlands shortly after delivery (LHL) and seven newborn lambs fully gestated at highlands but returned to lowlands shortly after delivery (HHL) were studied at 16 - 17 days-old (Fig. 1). Lung samples were taken to study both pulmonary artery morphometry through Van Gieson staining and PDGF-A, B, C and D through RT-PCR experiments.

**Results:** PDGF-A expression did not change in any of the experimental groups. However, LHL lambs showed greater expression of PDGF-B, compared to HHL or LLL, greater expression of PDGF-C compared to HHH and HHL, and greater expression of PDGF-D compared to HHH, HHL and LLL. The area of smooth muscle layer in the pulmonary artery wall was greater in LHL than in HHL and LLL group, whilst HHH group showed the greatest area of smooth muscle layer compared to the other groups.

**Conclusion:** PDGF-B, C and D may contribute to the pulmonary artery remodeling observed in newborn land exposed to perinatal chronic hypoxia. These PDGF isoforms could be potential therapeutic targets to revert pulmonary artery hypertension and remodeling associated to chronic hypoxia in the newborn.

*Supported by FONDECYT 1110595, 1120605, 1130424 and 1140647.*

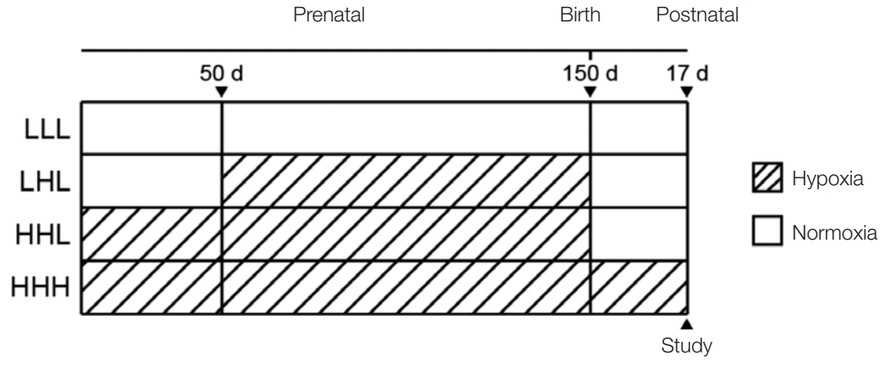


Figure 1. Different protocols of perinatal hypoxia exposure used in this study.

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P11

## Pulmonary anti-remodeling effects of melatonin on chronic hypoxic newborn sheep with pulmonary hypertension

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**Introduction:** Chronic hypoxia and oxidative stress during gestation lead to neonatal pulmonary hypertension (NPH)[1]. Main hallmarks of this disease are abnormal vasoreactivity and vascular remodeling[1,2]. Remodeling involves the expression of vasoactive determinants such as VEGF and smoothelin[3,4]. Melatonin has antioxidant and vasoactive properties and it's a potential therapeutic agent in perinatal medicine[5]. Therefore, we studied the vascular function and anti-remodeling effects of melatonin in NPH induced by hypoxia.

**Methods:** Bioethics Committee approved this study. 12 neonatal lambs were gestated in chronic hypobaric hypoxia and divided into two groups: 6 control (CN, EtOH 1.4%, 0.5 ml.kg$^{-1}$.d$^{-1}$ oral) and 6 melatonin treated (MN, 1 mg.kg$^{-1}$.d$^{-1}$ melatonin in EtOH 1.4%, 0,5 ml.kg$^{-1}$.d$^{-1}$ oral) between days 4-11 of life. At 12 days old lambs were euthanized and pulmonary samples were collected. We analyzed the contractile response to K$^+$ of pulmonary arteries by wire myography, smoothelin and VEGF expression by immunoblot and histological morphometry in the pulmonary vascular bed. The results were expressed as mean ± SEM and analyzed by t test ($p<0.05$).

**Results and discussion:** Melatonin treatment decreased the pulmonary contractile capacity and the vascular wall thickness. In addition, melatonin increased pulmonary vascular luminal area. Further, melatonin increased VEGF expression, but did not modified smoothelin expression (Fig. 1). Melatonin is modulating the vasoreactivity and enhancing the angiogenesis on the neonatal lung.

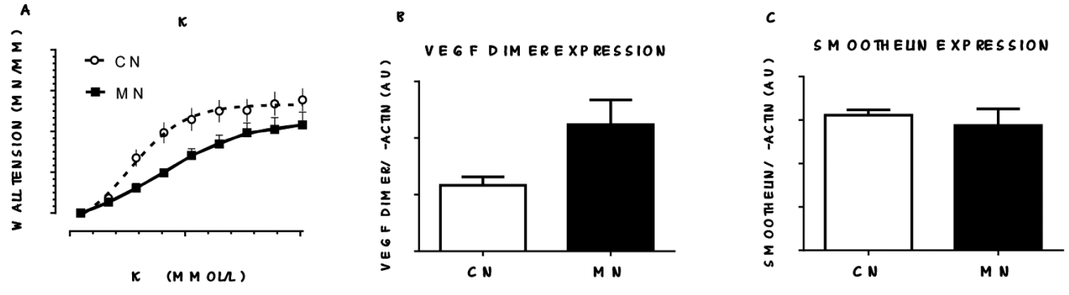


Figure 1. Melatonin vascular effects on pulmonary arteries. Contractile response to K+ (A), VEGF (B) and smoothelin (C) expression. Values are Mean ± SEM. Significant differences ($p<0.05$): * vs CN.

**Conclusion:** Melatonin treatment decreases the hypoxic remodeling processes in pulmonary arteries without a phenotypic differentiation of vascular smooth muscle cells. These beneficial effects support the therapeutic use of melatonin in the postnatal period.

*Supported by* FONDECYT 1110595, 1120605 & 1130424.

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5. Miller et al. *J Pineal Res*. 56:283-294, 2014.





P12

## A case of fetal parvovirus B19 myocarditis that caused terminal heart failure

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Parvovirus B19 is a well-established cause of fetal anemia and non-immune fetal hydrops in pregnancy. Fetal parvovirus infection can cause severe destruction of erythroid progenitor cells, resulting in fetal anemia, hydrops and intrauterine death. However, viral myocarditis with subsequent heart failure is another possible mechanism for hydrops formation as viral infection of fetal myocardial cells has been reported in post mortem examinations. We herein report a case of fetal cardiomegaly and massive pericardial effusion secondary to myocarditis as a result of parvovirus B19 infection. Doppler studies showed a high peak systolic velocity in the middle cerebral artery suggestive of fetal anemia. Fetal echocardiography showed cardiomegaly resulting in severe regurgitation of all valves and impaired ventricular function without structural cardiac defects. The endocardium was echo-dense, suggesting the presence of fibroelastosis. In view of the fetal circulatory disorder and lung decompression, it was decided to perform pericardial centesis and draining of the pericardial effusion at 22 weeks of gestation without any complications. However, the fetus died one day later, the fetus was died. Fetal pericardial fluid and ascites, as well as the amniotic fluid, tested positive for parvovirus B19 DNA and revealed a normal female karyotype 46, XX. Autopsy of the heart revealed severe hypertrophy and dilatation of the right and left ventricules. The dilated wall of the ventricule was almost circumferentially covered by a white scale of fibrous tissue and extensive inflammatory cell infiltrates were noted. The presence of endocardial fibroelastosis and myocarditis were confirmed by histology. The myocardial and hepatic tissues were investigated for parvovirus B19 RNA using polymerase chain reaction (PCR).
This case demonstrates that there may be an association between myocarditis caused by intrauterine parvovirus B19 infection and a poor outcome. The presence of viral myocarditis may be the determining prognostic factor in that situation.

P13

## Reduction of cardiac oxygen consumption during fetal heart rate decelerations: investigations with a simulation model

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It is hypothesized that fetal heart rate (FHR) decelerations contribute to a reduction in myocardial oxygen consumption during fetal hypoxemia [1,2]. Indeed, a reduction of FHR would reduce cardiac power, i.e. stroke work times FHR, if stroke work would remain constant. However, during FHR decelerations, the slowing of the heart will allow increased ventricular filling, thus increasing stroke work. Hence the net effect depends on the balance between the two.

Recently, a mathematical model has been developed to simulate FHR decelerations [3,4,5]. The model includes a feto-maternal circulation with explicitly modeled placenta (intervillous space and umbilical circulation) and fetal cerebral circulation. All other tissues are lumped within the fetal and maternal microcirculation compartment respectively. Oxygen pressures and concentrations are available for all feto-maternal compartments. The fetal circulation is under reflex regulation [6]. Uterine contractions can locally compress fetal or maternal blood vessels to induce different types of FHR decelerations [3,4,5]. To investigate whether FHR decelerations will reduce myocardial workload and thus myocardial oxygen consumption, the model was extended with a coronary circulation and myocardial oxygenation based on a relation between stroke work and oxygen consumption [7,8]. Cardiac oxygen metabolism at the nadir of variable FHR decelerations resulting from umbilical cord compression was compared to baseline level.

Simulation results show increased myocardial work (+15%) and oxygen consumption (+9%) per heart cycle. However, myocardial power is reduced by 33%. Reduced oxygen availability affects contractility and thus reduces myocardial work even more, by 45%.
The model results indicate that the fetus can indeed reduce myocardial oxygen consumption during FHR decelerations as the net result of reduced FHR, contractility and oxygen availability.

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P14

## Age-related changes and effects of mild hypothermia on carotid artery reactivity in newborn rats

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Therapeutic hypothermia has become a standard neuroprotective treatment in term newborn infants following perinatal asphyxia. Hypothermia-induced changes in the reactivity of the vessels supplying the brain might play a role in its therapeutic or side effects. We investigated the putative age-related changes and the effect of clinically relevant cooling (33°C) on the reactivity of the newborn rat carotid artery. Carotid artery rings from 2-3 d-old and 9-10 d-old rats were mounted in myographs and studied at 33 and 37°C. The contractions induced by KCl and U46619 and the relaxations induced by acetylcholine (ACh), the nitric oxide (NO) donor sodium nitroprusside (SNP), the NO-independent stimulator of soluble guanylate cyclase (sGC) BAY 41-2272, the β-adrenoceptor agonist isoproterenol, the adenylate cyclase activator forskolin, and acute hypoxia ($PO_2$ 3 kPa) were not significantly affected by the temperature. The relaxations induced by ACh, isoproterenol, the $\beta_2$-adrenoceptor agonist salbutamol, the $\beta_3$-adrenoceptor agonist CL-316243 and acute hypoxia increased with postnatal age and were impaired by endothelium removal or by inhibition of NO synthase (L-NAME) or sGC (ODQ). In contrast, the relaxations induced by SNP, BAY 41-2272 and forskolin were endothelium independent and did not change with age. In conclusion, mild hypothermia (33°C) does not affect the reactivity of neonatal rat carotid arteries. Our data suggest a reduced NO bioavailability in the carotid artery during the first days of life. This transient endothelial impairment might play a role in the adaptation of the circulatory system to birth and in the vascular response to insults such as hypoxia.





P15

## Protease-activated receptor (PAR)-mediated contraction of the chicken ductus arteriosus

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Department of Pediatrics, School for Oncology and Developmental Biology (GROW), Maastricht University Medical Center (MUMC+), Maastricht, Netherlands

**Background and aims:** PARs belong to a family of G protein– coupled receptors, thus mediating the cellular effects of proteinases. PAR1 and PAR2 have been shown to be involved in regulating vascular tone. Thrombin activates PAR1, whereas trypsin activates PAR1 and PAR2. Our aim was to evaluate the functional presence of PAR1 and PAR2 in the ductus arteriosus (DA).

**Methods:** We investigated, using wire myography, the mechanical responses induced by thrombin (0.1 to 3 U/mL), trypsin (0. 1 to 30 U/mL), the PAR1-activating peptide TFLLR- $NH_2$ (1 to 100 µmol/L) and the PAR2-activating peptide SLIGRL-$NH_2$ (0.1 to 10 µmol/L) in DA rings from 15-, 19-, and 21-d chicken embryos.

**Results:** Thrombin, trypsin, and TFLLR- $NH_2$, all caused concentration-dependent contraction of the pulmonary side of chicken DA. These contractions were not observed in the aortic side of the DA, in the femoral artery or in the pulmonary artery. Thrombin-, trypsin- and TFLLR- $NH_2$-induced contractions were endothelium-independent but markedly impaired by the elimination of calcium from the external medium. The contraction evoked by thrombin and trypsin increased between day 15 and 19 of incubation and was not affected by oxygen tension. SLIGRL-$NH_2$ (≥10 µmol/L), evoked endothelium-dependent relaxation of the DA.

**Conclusions:** PARs are functionally present in the chicken DA but not in other vascular tissues. Recent studies demonstrate that loss of platelet number or function leads to defective DA closure. We speculate that the role of platelets in DA closure might be partially mediated through the PAR-mediated vasoactive effects of thrombin.

P16

## Investigating the mechanisms underlying bronchopulmonary dysplasia using an in vitro cell stretch model

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Preterm infants often require respiratory support, which can injure the lungs and lead to abnormal lung development called bronchopulmonary dysplasia (BPD). There is no treatment for BPD because the mechanisms leading from lung injury to abnormal development are unknown. BPD is characterised by increased fibroblast proliferation, abnormal extracellular matrix deposition, inflammation, capillary dysplasia and impaired alveolarisation. Our aim is to mimic ventilation-induced lung injury (VILI) in cell culture to investigate the mechanisms leading to BPD.

Primary fetal rat lung fibroblasts were isolated during the canalicular stage of lung development at embryonic day (E)19 and exposed to 0 or 20% phasic over-distension. Cells were collected after 15 minutes and 1, 3, 6 or 24 hours to measure cell proliferation, inflammation (IL-1 , IL-6) and early markers of lung injury (CTGF, CYR61 and EGR1), by real-time PCR and/or immunohistochemistry. Data were analysed by ANOVA.

Fibroblasts exposed to 20% over-distension had increases in IL-1 (~2 fold), IL-6 (~6 fold), CTGF (~2 fold), CYR61 (~3 fold) and EGR1 (~9 fold) mRNA levels after 1h and an 11% increase in cell proliferation at 24 hours ($p<0.05$).

This in vitro model of over-distension injury successfully replicates many of the features associated with VILI including increased fibroblast proliferation, inflammation and markers of lung injury. This model will allow future studies to identify the cellular mechanisms and cell types that mediate the response of the lung to VILI and that may lead to BPD.





P17

## Effects of Holder pasteurization on the protein profile of human milk

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**Background:** Human milk (HM) has been identified as the optimal feeding to support growth and development of infants. When HM is not available, an alternative is donor milk (DM), which nevertheless requires a thermal treatment to ensure its microbiological safety. The most widespread method for the treatment of DM is the Holder pasteurization. The available literature data show that Holder pasteurization may cause degradation of some bioactive components, thereby potentially affecting the biological properties of HM. Nonetheless, the available data are scarce and conflicting, due to significant differences in the experimental protocols applied. The aim of this study was to determine the effect of Holder pasteurization on the protein profile of HM using a GeLC-MS method, which is able to identify both changes in the overall protein profile and specific proteins degraded by pasteurization.

**Methods:** Milk samples were collected by standardized methods from 20 mothers having delivered preterm and term, after obtaining informed consent. One part of each sample was immediately frozen, whilst another part was Holder pasteurized and then frozen. All samples were then analyzed by GeLC-MS. The protein bands of interest were excised from the gel, digested with trypsin and identified by nano-HPLC - MS/MS analysis.

**Results:** The protein profile before and after Holder pasteurization showed qualitative differences only in 6 samples out of 20, while in the remaining 14 no detectable differences were found. The differences were found to be associated with the decrease (or disappearance) of the electrophoretic bands corresponding to alpha and beta-casein, tenascin, lactoferrin and Immunoglobulin A.

**Conclusions:** Our findings support those previous studies that reported a small, but detectable, effect of Holder pasteurization on the HM protein profile. In the majority of samples, Holder pasteurization did not cause any modification, thereby preserving the biological activity of HM proteins.

P18

## Is umbilical artery dopplervelocimetry a predictor of feeding intolerance in preterm newborns?

Pace Carlotta [1], Gaglioti Pietro [1], Coscia Alessandra [1], Oberto Manuela [1], Cau Maria Antonietta [2], Palmeri Agnese [1], Appeddu Silvia [3], Olearo Elena [1], Plazzotta Claudio [4], Biolcati Marilisa [5], Todros Tullia [1]

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**Background:** Feeding intolerance (FI) is a possible precursor of necrotizing enterocolitis (NEC)[1,2], but still needs to be defined.
It is already known that fetal growth restriction (FGR) with abnormal umbilical artery Dopplervelocimetry (UAD) is significantly related to the development of NEC in preterm newborns[3,4]; we are investigating if it is also related to the development of FI.
In a previous study we analyzed all cases of NEC and FI diagnosed in our NICU from 2006 to 2012 (n=51): 100% of NEC (n=5/5) and 63% of FI (n=29/46) occurred in FGR fetuses with abnormal UAD.

**Methods:** We defined NEC according to Bell's modified criteria[5,6] and FI as a condition requiring discontinuation of enteral feeding for >24 hours[2].
We recruited all preterm fetuses (GA <35 weeks) born in our maternal-fetal care unit in 2013 (n=99).
We retrospectively categorized fetuses according to fetal growth/UAD (group A: AC<10°centile/retarded growth with abnormal UAD; group B: AC<10°centile/retarded growth with normal UAD; group C: AC>10°centile).

**Results:** We diagnosed 10 cases of FI and no cases of NEC; 70% of FI (n=7/10) occurred in FGR fetuses with abnormal UAD.
We recognized a statistically significant risk of developing FI in group A versus group C (OR 7.14 [95%CI 1.28-36.9]).
After stratification for gestational age, this feature was still observed for GA>28 weeks, although it was not significant probably due to the small number of cases. (Table 1)

**Conclusions:** The sensitivity of FGR with abnormal UAD in predicting FI reaches 70% in our study.
The involvement of splanchnic district is a consequence of centralization of blood flow in "brain sparing" FGR fetuses.
This prenatal condition should be signalled as probably requiring individualized nutritional care after birth.
The association between FGR/abnormal UAD and FI was not confirmed for extremely preterm newborns, probably because of the prevailing effect of low GA in this group.





Table 1.

| RISK OF FEEDING INTOLERANCE | OR | [95% CI] *significant |
|---|---|---|
| GROUP B vs C | 1.96 | [0.16-23.34] |
| GROUP A vs B | 3.64 | [0.40-32.84] |
| GROUP A vs C | 7.14 | [1.38-36.90] * |
| GROUP A vs C (GA<28w) | 0.42 | [0.01-14.08] |
| GROUP A vs C (GA 28+0-31+6w) | 6.8 | [0.66-69.63] |
| GROUP A vs C (GA>32w) | 17.08 | [0.82-355.44] |

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P19

## Oxygen-loaded nanodroplets counteract hypoxia effects on MMP/TIMP balances in human placental chorionic villi

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**Introduction:** Impaired trophoblast invasion is reportedly associated with poor placental perfusion and preeclampsia. Since matrix metalloproteinases (MMPs) and their physiological inhibitors (tissue inhibitors of metalloproteinases, TIMPs) play a crucial role during trophoblast invasion, the present study aimed at investigating hypoxia effects on secreted MMP/TIMP balances in placental chorionic villous explants from first-trimester pregnancies. Furthermore, the abilities of dextran-shelled decafluoropentane-containing oxygen-loaded nanodroplets (OLNDs) - a new class of oxygen nanocarriers recently developed by our group - to counteract hypoxia effects were also evaluated.

**Methods:** Small portions of placental chorionic villi (35 mg, n =3) from normal first-trimester pregnancies were cultured for 8 h at 37°C either in normoxic (20% $O_2$) or hypoxic (3% $O_2$) conditions in serum-free Ham's F12 medium with or without 10% v/v OLNDs. After supernatant collection, the protein levels of MMP-2, MMP-9, TIMP-1 and TIMP-2 were evaluated by complementary gelatin zymography and ELISA analyses.

**Results:** Normoxic explants released constitutively MMP-2 (~0.5 ng/ml) and MMP-9 (~4.5 ng/ml), as well as their respective inhibitors TIMP-2 (~9 ng/ml) and TIMP-1 (~22 ng/ml). Hypoxia significantly impaired MMP/TIMP balances by reducing the secretion of MMP-2, MMP-9 and TIMP-1, and by increasing that of TIMP-2. Dextran-shelled decafluoropentane-containing OLNDs - characterized by spherical morphology, ~550 nm diameters, and anionic surfaces - effectively abrogated all hypoxia effects, restoring either normoxic MMP or TIMP protein levels as well as MMP/TIMP balances.

**Conclusion:** Dextran-shelled decafluoropentane-containing OLNDs proved effective in counteracting hypoxia effects on the balances between MMPs and TIMPs secreted by human placental chorionic villous explants from first-trimester pregnancies. Therefore, they appear as promising, innovative and low-cost therapeutic nanodevices to treat hypoxia-associated placental disorders, including preeclampsia.





P20

## Characterization of human placenta-derived stem cells in intrauterine growth restriction

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**Introduction:** Placental mesenchymal stem cells (pMSC) have been recently investigated in preeclamptic placentas, suggesting their alterations may originate in the stem cell compartment. However, pMSC have never been studied in Intrauterine Growth Restriction (IUGR).
The aim of this study was to isolate, characterize and compare human IUGR and normal pMSC.

**Methods:** We isolated cells from both the placental disc membranes (fetal-side) and villous mesenchyma of 12 human single pregnancies placentas at elective cesarean section: 6 low-severity IUGR, 5 term-controls (AGA), 1 pre-term AGA.
Viability and proliferation: assessed every 7 days over a 6-week culture;
Cell type evaluation:
hematopoietic (CD133,CD34,CD45,CD117), mesenchymal (CD105,CD29,CD44,CD73,CD90) and endothelial (CD31,CD31/146/90) markers expression by flow cytometry (24 hours, 7 days, 30 days).
Differentiation potential: cell culture in appropriate media for adipogenic, osteogenic, chondrogenic, myogenic and endothelial differentiation. Quantitative evaluation of adipogenic, endothelial and myogenic differentiations by spectrofluorimetry (Oil-Red-O) or counting of FactorVIII/desmin-alphaSMA-positive cells.

**Results:** High cell viability over 6 weeks.
Samples presented a starting heterogeneous population, progressively increasing mesenchymal markers. This occurred earlier in IUGR than in term/pre-term AGA: at day 7 mesenchymal markers were 1.6-4 fold (fetal-membranes) and 2.3–5.8 fold (villous trees) higher in IUGR.
pMSC from both AGA and IUGR differentiated into tested lineages. IUGR showed significantly higher adipogenic and lower endothelial differentiation potential vs pre-term/term AGA.

**Conclusions:** Mesenchymal markers enrichment and multipotent differentiation abilities confirmed successful pMSC isolation and selection.
IUGR-specific features may suggest a role of pMSC in IUGR pathogenesis. However, they might also be consequence of altered placental environment influencing pMSC.
We might speculate that differences were not related to IUGR early gestational age, as pre-term AGA did not differ from term AGA for overall analysis. Further pre-term AGA analysis are needed to confirm it.
pMSC mitochondrial content and cytokine analysis are in progress to evaluate oxidative stress and inflammatory status.

P21

## Effect of Bisphenol A on endometrial and placental physiology

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**Objectives:** Bisphenol A (BPA) is an widely diffuse environmental compounds which may influence human reproductive health. Women in reproductive age and mothers during pregnancy can be daily exposed to BPA. In the present study we investigated the effect of BPA on tissue models representative of the feto-maternal interface, namely the human endometrium and placenta under *in vitro* exposure.

**Methods:** Stromal cells were isolated from healthy endometrial tissues and *in vitro* decidualized. Cells cultures were than exposed to 1 nM BPA for 24 hours. The protein and mRNA expression of Estrogen (ER , ERβ), Progesterone (PRA, PRB), and human Chorionic Gonadotropin/ Luteinizing Hormone (hCG/LH) receptors was investigated by western blot and real time PCR.
Placenta explants were isolated from human placenta and cultured on matrigel. The villous explants were exposed to 1 nM BPA for 24 and 48 hours and the secretion of β-hCG was measured by ELISA.
Finally placental cultures were exposed to conditioned medium collected from endometrial cells treated or not with BPA. The effect of endometrial-conditioned medium on β-hCG secretion was investigated by ELISA assay.

**Results:** We found that BPA up-regulated ERs and PRs while it down-regulated hCG/LHR expression both at mRNA and protein level in endometrial cells. Furthermore, BPA induced the secretion of β-hCG by the placenta. The exaggerated secretion of β-hCG observed in placental cultures exposed to BPA was abolished when placental explants were cultured with endometrial-conditioned medium.

**Conclusions:** Altogether, these data show that BPA exposure influences endometrial and placental physiology and highlight the importance of *in vitro* models including the maternal component in reproducing the effects of environmental chemicals on human fetus/placenta.





P22

## Low Molecular Weight Heparin (LMWH) inhibits placenta inflammation by tuning HMGB1's affinity for RAGE receptor

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**Objectives:** Preeclampsia (PE) is a severe pregnancy-related syndrome characterized by exacerbated placental inflammation. Recent data described LMWH's anti-inflammatory action effective in PE treatment. Nevertheless, LMWH mechanism of action on the placental tissue is still unclear. We previously demonstrated that LMWH is able to increase High Mobility Group Box 1 (HMGB1), transcription factor with extracellular cytokine-like function, in placental villous explants. In stark contrast, we found a down-regulation of IL-6 and TNF alpha, two main pro-inflammatory cytokines whose transcription is stimulated by HMGB1 binding to its receptor RAGE (Receptor for Advanced Glycation End Products). In the present study we investigated whether LMWH could modify RAGE expression and/or its affinity for HMGB1.

**Methods:** Human placental villous explants (n=32) were excised from physiological placentae (n=4), cultured in HAM F12 medium and treated for 24h by LMWH 0.5 units (U; Parnaparin, Alfa Wassermann, Italy) or plain culture medium (controls). RAGE protein expression was determined by Western Blot assay. RAGE/HMGB1 binding affinity was assessed by RAGE immunoprecipitation (IP) followed by HMGB1 immunoblot performed on the resulting pellets and RAGE-deprived placental lysates.

**Results:** We reported no differences in RAGE protein levels in placental villous explants treated by 0.5U LMWH compared to untreated controls. In contrast, HMGB1 significantly decreased in the IP pellet ($p=0.022$) and it increased in the RAGE-deprived lysates ($p=0.003$) of LMWH-treated villous explants compared to controls.

**Conclusions:** Increased free HMGB1 in the LMWH-treated placental lysates after RAGE IP strongly suggest an heparin-mediated HMGB1 conformation change able to diminish its affinity for RAGE, as previously demonstrated in other systems. Our data could explain the placental HMGB1 accumulation accompanied by TNF alpha and IL-6 decreases that we previously reported. We provided new insights on the LMWH's anti-inflammatory effect clinically observed in PE women.

P23

## Gestational diabetes mellitus (GDM) modulates human placental apoptosis

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**Objectives:** Gestational diabetes mellitus (GDM) is a common complication in pregnancy, affecting more than 7% of pregnancies worldwide with high risks for both mother and fetus. It is known that a finely tuned trophoblast apoptosis is pivotal for normal placental development and homeostasis. Indeed, imbalance of programmed cell death may alter placental function and afterward gestational success. In particular, it has been suggested that activation of poly(ADP-ribose) polymerase-1 (PARP-1), caspase 3 and the leucine zipper, down-regulated in cancer (LDOC) genes, important mediators of the apoptotic process, play important roles in the patho-physiology of GDM. Herein, we investigated the expression of PARP-1, caspase 3 and LDOC1 in placentae from GDM pregnancies.

**Methods:** Placental biopsies were immediately collected after delivery from GDM (n=10) and control (CTRL, n=10) pregnancies. PARP-1, caspase 3 and LDOC mRNA expression was assessed by Real Time PCR while their protein levels were determined by Western Blot.

**Results:** We found that PARP-1, caspase 3 and LDOC mRNA expression were significantly increased in GDM vs CTRL placentae ($p<0.05$). Gene expression data were confirmed at the protein level by western blot ($p<0.05$).

**Conclusions:** Herein, we demonstrated that PARP-1, caspase 3 and LDOC were over-expressed in GDM placentae. Our data suggest that diabetes may be a clinical condition triggering placental apoptosis thus altering gestational physiology. Indeed, regulation of PARP-1, caspase 3 and LDOC expression within GDM placentae may constitute a target for therapeutic approaches in order to prevent the adverse effects of GDM on women and their offspring.





P24

## Fine particulate matter in the air within the limits recommended by WHO alters placental structure and circulating and local Renin Angiotensin System

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**Objective:** To evaluate the consequences of exposure to air pollution on placental structure.

**Methods:** Female Wistar rats (7-9/group) were exposed to filtrated air (F) or particulate matter (P) in a concentrator of atmospheric particles for 15 days. After mating, at the 6th day of pregnancy, rats were divided in 4 groups and again exposed to F or P (FF, FP, PF, PP). At the 19th day of pregnancy, placentas (fetal portion (fPl) and maternal portion (Dc)) and fetus were collected. Several placental parameters were evaluated as mass, thickness, surface area (as from longitudinal and transversal diameters measured with a digital caliper), fractional area, and protein expression of TGF  and renin angiotensin system (indirect ELISA).

**Results (mean±SEM – p<0.05):**

| | | FF | FP | PF | PP |
|---|---|---|---|---|---|
| ***Mass (g)*** | **fPl+Dc** | 0.63±0.010 | 0.61±0.009 | 0.57±0.014[1] | 0.55±0.007[1] |
| | **fPl** | 0.42±0.006 | 0.44±0.006 | 0.40±0.005[1,2,4] | 0.43±0.004 |
| | **Dc** | 0.13±0.005 | 0.14±0.008 | 0.12±0.005[1,2] | 0.12±0.007[1,2] |
| ***Thickness*** | **fPl** | 4.42±0.10 | 4.16±0.06 | 3.93±0.09[1,4] | 4.40±0.11 |
| ***(mm)*** | **Dc** | 3.15±0.10 | 2.77±0.09[1] | 2.92±0.09 | 2.95±0.11 |
| ***Surface area (mm²)*** | **fPl** | 117.6±2.37 | 90.20±2.19[1] | 101.1±2.97[1,2] | 101.7±2.28[1,2] |
| ***(LDxTDxπ)/4*** | **Dc** | 32.64±1.46 | 24.0±0.60[1] | 28.4±1.11[1,2] | 26.73±1.46[1] |
| ***ACE activity*** | **fPl** | 1.05±0.07 | 0.99±0.11 | 1.12±0.10 | 1.35±0.24 |
| ***(nmol His-*** | **Dc** | 0.83±0.07 | 0.67±0.06 | 0.59±0.05[1] | 0.53±0.05[1] |
| ***leu/min/mL)*** | **Serum** | 33.50±1.39 | 28.89±1.37[1] | 28.90±1.67[1] | 26.53±1.06[1] |
| **Renin activity** ***(ng/mL/hour)*** | **Plasma** | 24.82±1.83 | 30.54±1.399[1,4] | 31.97±0.799[1,4] | 21.69±3.109 |
| **ACE** | **fPl** | 100±0.125 | 90.17±5.270 | 74.20±4.839[1,2] | 59.78±3.364[1,2] |
| **(%)** | **Dc** | 100±0.14 | 96.96±4.391 | 82.04±3.123[1,2] | 80.41±4.964[1,2] |
| **AT1R** | **fPl** | 100±0.142 | 202.1±41.16[1] | 118.7±17.69 | 142.3±15.91 |
| **(%)** | **Dc** | 100±0.142 | 99.71±11.19 | 75.58±5.210 | 36.63±4.019[1,2,3] |

| | | | | | |
|---|---|---|---|---|---|
| **AT2R** | **fPl** | 100±0.142 | 97.06±8.512 | 63.69±11.22[1,2] | 39.46±7.537[1,2] |
| **(%)** | **Dc** | 100±0.142 | 91.10±7.968 | 73.86±10.37 | 39.77±7.101[1,2] |
| **TGFβ** | **fPl** | 100±0.142 | 76.93±8.581 | 76.63±3.763 | 83.30±5.962 |
| **(%)** | **Dc** | 100±0.142 | 72.70±6.355 | 48.58±5.905 | 49.54±5.770 |

$p<0.05$ [1]vs FF; [2]vs FP; [3]vs PF; [4]vs PP. TD and LD=transversal and longitudinal diameter.

**Conclusions:** PM exposure affects placental structure, placental renin angiotensin system and alters the activity of key-enzymes of this system with a possible prejudice of transference of nutrients from mother to fetus.





P25

## Folate transporters expression in placentas related to birth weight and concentrations of folates in cord blood

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Adequate nutrition during gestation is essential for normal development and growth. In general, a positive correlation between folate intake during pregnancy and birth weight has been shown. However, a high supply of FA through fortified foods and supplements, along with the observation that an excessive consumption of FA in the third trimester of pregnancy was associated to a lower birth weight, has increased the interest in studying the effects of excessive folic acid (FA) consumption during pregnancy. At present, the relationship between placental folate transporters and birth weight is unknown. In this study, we selected 107 placentas of term infants with different birth weight: appropriate (AGA, n=41), small (SGA, n=33) and large (LGA, n=33) for gestational age. In placental tissue we determined the gene expression (mRNA) and protein content of folate transporters (FOLR1, RFC1 and HCP1) by real time PCR (qRT-PCR) and western blot (WB) respectively. Also, in a subgroup of neonates, the concentration of folates in cord blood was measured by electrochemiluminiscence. The results show that SGA infants have a higher concentration of folates than the AGA and LGA (p 0.05), which was related to a lower expression in mRNA of rfc1 (p=0.02) in representative samples of total placental tissue. Protein concentration of folate transporters was studied separately in basal (maternal side) and chorionic plates (foetal side) of placental tissue. A lower FOLR1 and RFC1 concentrations were found in the chorionic plate and basal plates of the SGA and LGA placentas respectively, compared to the AGA group. HCP1 remained unchanged in all groups. This study suggests that excessive folate levels in newborns could be negatively related to birth weight. Amounts of folates intake and its supplementation period during pregnancy should be reviewed and optimized to prevent foetal developmental alterations and thus avoiding long term consequences in health.

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P26

## Evidences that high- or low-salt intake during pregnancy does not alter kidney structure in the newborns

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**Objective:** This study aims to evaluate a possible influence of high- or low- salt diet during pregnancy on renal structure in neonates of Wistar rats.

**Methods:** Pregnant rats were fed with normal, high or low-salt diet (NR, HO and HR groups, respectively) from the first day of gestation until delivery. Kidneys of the newborns were collected in the first 24 hours of life. Total renal volume and its compartments (cortex, medulla and pelvis) were evaluated by stereology.

**Results:**
**Table 1.** Total kidney volume and its compartments of newborn females and males (mean±standard; n = 6/group).

| | Newborn | HO | NR | HR |
|---|---|---|---|---|
| Total kidney ($mm^3$) | Males | 7.04±0.47 | 7.44±0.22 | 8.20±0.46 |
| | Females | 6.73±0.61 | 5.84±0.44 | 7.25±0.98 |
| Cortex ($mm^3$) | Males | 4.97±0.34 | 5.40±0.16 | 5.86±0.27 |
| | Females | 4.74±0.48 | 4.19±0.28 | 4.99±0.61 |
| Medulla ($mm^3$) | Males | 1.89±0.16 | 1.87±0.07 | 2.16±0.16 |
| | Females | 1.76±0.15 | 1.51±0.16 | 2.08±0.36 |
| Pelvis ($mm^3$) | Males | 0.18±0.04 | 0.17±0.02 | 0.18±0.06 |
| | Females | 0.22 ±0.03 | 0.14±0.04 | 0.18±0.04 |
| Cortex volume fraction (%) | Males | 0.71±0.01 | 0.73±0.00 | 0.72±0.01 |
| | Females | 0.70±0.01 | 0.72±0.02 | 0.70±0.01 |
| Medulla volume fraction (%) | Males | 0.27±0.01 | 0.25±0.01 | 0.26±0.01 |
| | Females | 0.26±0.01 | 0.26±0.02 | 0.28±0.01 |
| Pelvis volume fraction (%) | Males | 0.03±0.01 | 0.02±0.00 | 0.02±0.01 |
| | Females | 0.04±0.01 | 0.02±0.00 | 0.03±0.01 |

**Conclusion:** Kidney structure of newborns was not affected by different low-or high-salt intake during pregnancy.





P27

## Changes in fetal heart rate patterns in preterm prelabor rupture of membranes with and without histological chorioamnionitis

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**Introduction:** Preterm prelabor rupture of membranes (pPROM) complicates 1-2% of all pregnancies. Although the etiology is multifactorial, an infective mechanism is thought to be involved in the majority of cases. The clinical management of pPROM depends on gestational age and signs of fetal infection. Invasive procedures such as amniocentesis have been used to diagnose intra-amniotic infection. Cardiotocography (CTG), a non invasive test for monitoring fetal well-being, may provide useful information to detect fetal infection and thus help to decide timing of delivery.

**Objective:** To examine the association between CTG abnormalities and acute histological chorioamnionitis (as a gold standard for intrauterine infection) in the presence of pPROM.

**Methods:** The study population consisted of 130 pregnant women with pPROM between 28+0 and 34+0 weeks of gestational age divided into acute histological chorioamnionitis (HCA) and normal placenta (Controls) based on postpartum histological evaluation of the placenta and membranes. The last antepartum FHR tracing was evaluated; multivariate logistic regression was used for analysis. Statistical significance was set at $p<0.05$.

**Results:** After adjustment for gestational age, latency between pPROM and delivery, multiple pregnancy, maternal age and BMI, HCA was significantly associated with FHR variability <5 bpm [OR 8,20; 95% CI 2.31-29.08] and the presence of decelerations [OR 2,37; 95% CI 1.07-5.23]: after subcategories analysis only variable decelerations were significantly associated with HCA [OR 3,12; 95% CI 1,24-7,71]. No difference between HCA and Controls were found in baseline FHR, presence and number of accelerations, and neonatal outcomes.

**Conclusion:** In the presence of pPROM between 28 and 34 weeks of gestational age, minimal or absent FHR variability (<5 bpm) and/or variable decelerations may represent a sign of intra-amniotic infection.

P28

## The Perinatologists' family tree - Reloaded

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At the 2013 FNPS meeting in Chile, we presented an interactive poster to develop a family tree, based on the researchers in perinatal medicine, their histories and connections.
The current worldwide research in Perinatal Physiology and Pathophysiology is, without a doubt, part of our legacy, starting with Sir Joseph Barcroft and including the current students learning at our Labs.
This is a new interactive abstract and poster, not meaning to describe the absolute hierarchy of the individuals in the history of perinatology, but to develop and sprout up the tree with the actual characters.
We encourage you to visit this poster and add to it, creating part of the history in the Reloaded Perinatologists' Family Tree.

*Supported by* many researchers throughout history.





P29

## Do SGA fetuses have less reserves in labour than AGA fetuses? A retrospective study

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**Introduction:** The aim of the present study is to test the hypothesis that Small for Gestational Age fetuses (SGA) are more subjected to develop pathological cardiotocography (CTG) tracings during labour and that the time from the beginning of labour and the advent of a pathological CTG tracing is shorter in SGA than in normal weight fetuses.

**Methods:** The study was conducted at Maternal-Fetal Medicine Unit of Sant'Anna University Hospital in Turin, Italy.
We retrospectively analysed CTG and clinical records of 355 SGA (96 cases < 3$^{rd}$ centile;
259 cases ≥ 3$^{rd}$<10$^{th}$ centile) who were born at term between January and December 2012 and we compared them to those of a control group (575 cases) characterized by appropriate for gestational age (AGA) neonates born at term.
Tracings were evaluated independently by two obstetrics consultants according to the FIGO classification.
The main outcomes considered were the incidence of abnormal CTG and the time interval between the beginning of labour and the advent of pathological CTG.

**Results:** SGA more frequently presented pathological CTG in labour than controls (39.1% versus 21.6%; p<0.001). In particular, SGA < 3$^{rd}$ centile had a pathological CTG rate of 43.8%, while SGA ≥ 3$^{rd}$ < 10$^{th}$ centile had a rate of 37.4% with a significant difference in both groups compared to controls (p<0.001). The develoopement of pathological CTG was faster in the SGA group, with an increasing trend from SGA < 3$^{rd}$ centile to the control group (p< 0.05 – Table 1).

**Conclusion:** The results confirm the hypothesis that SGA would have less reserves to deal with labour. SGA neonates should be followed in during childhood to compare long term outcomes with AGA.

| | SGA < 3rd centile (96 cases– group1) | SGA ≥ 3rd < 10th centile (259 cases– group2) | All SGA (355 cases– group3) | Controls (575 cases) | P value | Test |
|---|---|---|---|---|---|---|
| **Sex (M--F)** | 49.0%--51.0% | 56.8%--43.2% | 54.7%--45.3% | 53.8%--46.2% | NS | c2 |
| **Pathological CTG** | 42 (43.8%) | 97 (37.4%) | 139 (39.1%) | 124 (21.6%) | p1 = NS<br>p2-p3-p4<0.001 | c2 |
| **Patterns more frequent in pathological CTG** | Severe variable/late decelerations<br>36/42 (85.7%) | Severe variable/late decelerations<br>79/97 (81.4%) | Severe variable/late decelerations<br>115/139 (82.7%) | Severe variable/ late decelerations<br>108/124 (87.1%) | NS | c2<br>Fisher |
| **Minutes from labour begining and pathological CTG (median, range)** | 53 (0-277) | 130 (0-555) | 118 (0-555) | 170.5 (0-550) | p1-p2-p3 <0.05<br>p4 <0.001 | Mann-Whitney |
| **Minutes from pathological CTG and delivery (median, range)** | 67.5 (13-200) | 55 (8-200) | 59 (8-200) | 50 (7-306) | NS | Mann-Whitney |
| **Vaginal delivery** | 74 (77.1%) | 207 (79.9%) | 281 (79.1%) | 463 (80.6%) | NS | c2 |
| **Caesarean section** | 16 (16.7%) | 33 (12.7%) | 49 (13.8%) | 73 (12.7%) | NS | c2 |
| **VEM** | 6 (6.2%) | 19 (7.4%) | 25 (7%) | 38 (6.7%) | NS | c2 |
| **Gestational age at delivery (media ± DS)** | 39.3 ± 1.1 | 39 ± 1 | 39.1 ± 1.1 | 39.4 ± 1.1 | p1 <0.05<br>p2 =NS<br>p3 <0.001 | T Student Indipendent |
| **Minutes of labour (median, range)** | 136.5 (40-603) | 172 (5-635) | 160 (5-635) | 200 (23-840) | p1 <0.05<br>p2-p3-p4 <0.001 | Mann-Whitney |
| **Apgar<7 at 5'** | 3 (3.1%) | 0 | 3 (0.8%) | 2 (0.3%) | p1-p2<0.05<br>p3-p4 = NS | Fisher |
| **Lactate suggestive for acidosis** | 5/52 (9.6%) | 7/119 (5.9%) | 12/171 (7%) | 15/214 (7%) | NS | c2,<br>Fisher |
| **Neonatal weight (media ± DS)** | 2435.9 ± 268 | 2664 ± 142 | 2587.6 ± 221 | 3456.4 ± 327.4 | p1-p2-p3-p4 <0.001 | T Student |
| **SGA not diagnosed prenatally (unrecognized)** | 72 (75%) | 240 (92.7%) | 312 (87.9%) | / | p1 <0.001 | c2 |
| **Neonatal resuscitation** | 2 (2.1%) | 9 (3.5%) | 11 (3.1%) | 6 (1%) | p1-p2=NS<br>p3-p4<0.05 | c2<br>Fisher |

Table 1: Results - Legend: p1 = group1 versus group2; p2 = group1 versus controls; p3 = group2 versus controls; p4= group3 versus controls





P30

## The role of perinatal medical center for treating HIV-infected pregnant women with premature delivery in Japan

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**Objectives:** Facilities that are able to treat premature delivery of HIV infected women are not prevalent because such facilities need to treat both premature delivery and HIV infection which is relatively rare in Japan. Our study focused on current status of perinatal centers for treating premature delivery of HIV infected women in Japan.

**Materials & methods:** Survey sheets were sent by mail to general and regional perinatal centers and AIDS core hospitals with obstetrics department. Questionnaire includes capability of delivering HIV infected women and the earliest treatable gestational age. 75% of general and 41% of regional perinatal centers was assigned to AIDS core hospital.

**Results:** Of the 413 facilities 353 answered the questionnaire. Fifty nine general perinatal centers were assigned to AIDS core hospital and 50 (84.7%) facilities either delivered HIV infected women in the past or are possible to deliver HIV infected women. All except 2 had no lower limit of gestational age. Although 9 facilities were impossible to deliver HIV infected women, they recognize nearby facility capable of delivering HIV infected women. Of 14 general perinatal centers without AIDS core hospital assignment, 5 were capable to deliver HIV infected women. Sixty three (70.8%) regional perinatal centers with AIDS core hospital assignment were capable to deliver HIV infected women (15 facilities had no lower limit of gestational age).

**Conclusion:** General perinatal centers are supposed to treat very premature delivery and/or severe obstetrics complications and are installed at least 1 per each prefecture, supplemented with regional perinatal centers. Region with general perinatal center which is capable of delivering HIV infected women may have no problem to treat HIV infected pregnant women with premature delivery. Cooperative system has to be established in the region without general perinatal center which is capable of delivering HIV infected women.

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## Predictive factors for Mirror Syndrome and evaluation of neonatal outcomes in a population of fetuses with effusion

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**Objective:** The aim of our study was to identify predictive factors for Mirror syndrome[1], a rare syndrome defined by maternal edema, fetal hydrops and placentomegaly, and to analyze adverse neonatal outcomes in fetuses with effusion.

**Methods:** We retrospectively reviewed 45 maternal and 49 neonatal records(4 twin pregnancies) of newborn and stillbirths with effusion, from 2006 to 2014. Fetal hydrops was defined as abnormal fluid in at least two fetal compartments. Polyhydramnios and placentomegaly were considered. Maternal symptoms were edema, excessive weight-gain, hypertension, pulmonary edema, mild hemodilution, elevated liver enzymes, proteinuria. Three groups were defined: 1)asymptomatic women, 2)women presenting at least one symptom with placentomegaly or hydrops ("mirror syndrome-like"MSL), 3)mirror syndrome(MS). To evaluate predictive factors for MS and MSL, OR for fetal and maternal characteristic were calculated. Short-term neonatal outcomes were evaluated.

**Results:** Predictive factors for MS were fetal tricuspidal regurgitation, hydrops+polyhydramnios, hydrops with ascites or hydrothorax. A predictive factor for MSL was polyhydramnios. Perinatal mortality was similar among the 3 groups(Table1). Patients with MS and MSL underwent the same rate of intrauterine interventions(intrauterine transfusion, fetal paracentesis, thoracentesis, evacuative amniocentesis) compared with asymptomatic ones; however, infants who received intrauterine treatments were more often born alive (60,95%vs30,4%, p0,037). Birthweight standard-deviation-score was higher in MS compared with asymptomatic mothers. Neonates of the MS and MSL group had more often Apgar score<4 at 1'(but not at 5'), more frequent intubation and ventilation compared with the neonates of asymptomatic mothers, nevertheless the GA at delivery was similar between MS+MSL and asymptomatic group.

**Conclusions:** The number and type of compartments of fetal effusion could be a predictive factor for MS. However, in our case-serie, MS was not associated with increased rate of perinatal mortality. Our data suggest that intrauterine intervention should be performed to improve fetal outcomes, but, if the etiology of effusion remains unclear, intrauterine therapies are not always useful to prevent maternal symptoms.

*References*

1. Braun T. et al. Mirror Syndrome: A Systematic Review of Fetal Associated Conditions, Maternal Presentation and Perinatal Outcome, Fetal Diagn Ther 2010;27:191–203





Table 1 Pregnancy and neonatal outcomes, predictive factors for MS and MSL

P1= Asymptomatic Vs MS; P2= Asymptomatic Vs MSL ; P3=MSL Vs MS; P4=MS+MSL Vs asymptomatic, OR1 for MSL, OR2 for MS

| | **Non Mirror N 21** | **Simil Mirror N 10** | **Mirror Syndrome N 14** | **P value** |
|---|---|---|---|---|
| GA at diagnosis of effusion (weeks) Median (Min-Max) | 25 (19-37) | 30 (19-36) | 27,5 (24-33) | P1= 0,43 P2= 0,4 P3= 0,44 |
| GA at delivery (weeks) Median (Min-Max) | 32 (20-39) | 34 (30-37) | 29 (25-36) | P1= 0,31P2= 0,39 **P3= 0,007** P4=0,17 |
| Δ GA at diagnosis of effusion/ GA at delivery (weeks) Median (Min-Max) | 2 (0-19) | 3 (0-13) | 0.5 (0-7) | P1=0.24 P2= 0.67 P3= 0.13 |
| Cesarean Section N (%) | 6 (28,6%) | 7(70%) | 11 (78,6%) | **P1= 0.01** P2= 0.052 P3= 1.00 |
| Birthweight SDS Median (Min-Max) | 0.31 (-2.31/3.91) | 1.06 (-1.18/8.54) | 2.30 (0.12/8.17) | **P1= 0,002** P2= 0,23 P3= 0,11 |
| Alloimmune etiology N (%) | 3 (14,3%) | 1(10%) | 2(14,3%) | P1= 1,0 P2= 1,0 P3= 1,0 |
| Non allo- immune etiology N (%) | 18(85,7%) | 9(90%) | 12(85,7%) | |
| Intrauterine interventions (transfusion, thoracentesis, paracentesis, evacuative amniocentesis) N (%) | 7(33%) | 5(50%) | 8 (57,1%) | P1=0,29 P2=0,44 P3=1,0 |
| Perinatal death N (%) | 14(63,6%) | 6 (60%) | 8 (57,1%) | P1=0,7 P2=1,0 P3=1,0 P4= 1,0 |
| Apgar score <4 at 1' | 0 | 4 | 6 | **P1=0,007 P2=0,02** P3=0,60 **P4= 0,003** |
| Apgar score <4 at 5' | 0 | 2 | 2 | P1= 0,46 P2=2 P3=1 P4=0,25 |
| Intubation in the delivery suite N(%) | 0 | 3 | 6 | **P1=0,001** P2=0,07 P3=0,26 **P4= 0,006** |
| Mechanical ventilation N(%) | 2 | 4 | 6 | **P1=0,04** P2=0,27 P3=0,55 **P4= 0,032** |
| | | | | **OR1 for MSL OR2 for MS (CI 95%)** |
| Maternal age >33 years N | 8 | 7 | 6 | OR1 3,79 (0,75-19) OR2 1,22 (0,31-4,83) |
| GA at diagnosis of fetal effusion > 27 weeks N | 9 | 6 | 7 | OR1 2,0 (0,43-9,26) OR2 1,33 (0,34-5,18) |
| Fetal tricuspidal regurgitation N | 3 | 3 | 6 | OR1 2,0 (0,27-14,78) **OR2 8,0 (1,01-63,96)** |
| Ascites N | 9 | 5 | 9 | OR1 1,33 (0,29-6,04) OR2 2,4 (0,59-9,67) |
| Hydrothorax N | 7 | 4 | 7 | OR1 1,33 (0,28-6,32) OR2 2,0 (0,50-7,99) |
| Polyhydramnios N | 3 | 6 | 6 | **OR1,9 (1,55-52)** OR2 4,5 (0,89-22) |
| Hydrops with ascites N | 3 | 3 | 8 | OR1 2,6 (0,41-16) **OR2 8 (1,58-40,3)** |
| Hydrops with hydrothorax N | 3 | 4 | 7 | OR1 4 (0,69-23,22) **OR2 6 (1,2-30)** |
| Hydrops with polyhydramnios N | 1 | 3 | 5 | OR1 8,57 (0,76-96) **OR2 ,11 (1,12-109)** |

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