Fetal and Neonatal Physiological Society

39th Annual Meeting

8th to 11th July 2012

Woudschoten, Zeist, The Netherlands

Conference Organising Committee

Jan Derks
Joepe Kaandorp
Eduard Mulder
Deodata Tijsseling
Gerard Visser
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General Information

Utrecht
Utrecht is the capital of the Dutch province of Utrecht, and is the fourth largest city of the Netherlands. Utrecht's ancient city-centre features many buildings and structures from the early Middle Ages. It has been the religious centre of the Netherlands since the eighth century. Until the golden age, Utrecht was the city of most importance in the northern Netherlands (the present-day country of the Netherlands), until Amsterdam became the cultural and most populous centre of the Netherlands. Utrecht is host to Utrecht University, the largest university of the Netherlands, as well as several other institutes for higher education. Due to its central position within the country it is an important transportation hub. It has the second highest number of cultural events in the Netherlands, after Amsterdam.

Utrecht Tourist Information
VVV Utrecht
Domplein 9
3512 JC UTRECHT
Tel.:0900-1288732
infovvv@toerisme-utrecht.nl
www.bezoek-utrecht.nl

Electricity
Electricity in the Netherlands is supplied at 230V-50Hz.

Currency
The currency is the Euro. The majority of hotels, restaurants and shops accept credit cards.

Weather
July is summer time in the Netherlands, so be prepared for sunshine and rain!

Conference Venue
Woudschoten Conferentiecentrum
Woudenbergseweg 54
3707 HX Zeist
Phone +31 (0)343 - 492 492

Conference website
http://www.fnps2012.nl/

Parking
Free parking is nearby the conference venue.

Language
The official language of the conference is English.
Name Badge
All participants, accompanying persons and exhibitors must wear the conference identification badge. Entrance to the meeting hall, poster and exhibition area, and the restaurant will not be permitted to any person without a badge. Do also wear your badge during outdoor activities.

Internet Access
WiFi is available throughout the conference venue.

Trade Stands
Representatives of companies which made the conference financially possible will be present during the meeting. Please make an effort to visit their stands.

Disclaimer
The 39th FNPS meeting and/or its agents have the right for any reason beyond their control to alter or cancel, without prior notice, the conference or any part of the arrangements, timetables, plans or other items relating directly or indirectly to the 39th FNPS meeting. The organization shall not be liable for any loss, damage, expenditure or inconvenience caused as a result of such alteration or cancellation. In addition, participants shall arrange their own medical and travel insurance.

Conference Management
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E info@fnps2012.nl
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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 investigators in training, 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 (1929-2010) 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:
1974-1980 Conference on Fetal Breathing
1981-1983 International Conference on Fetal Breathing and other Movements
1984-1995 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 US$.
The (local) Organizational 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 fellows-in-training to attend the meeting of the society.
Previous Meetings of the FNPS

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<th>Year</th>
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FNPS Board Members 2012

Jan Nijhuis, Chair, The Netherlands
Dino Giussani, Scribe, United Kingdom
Emilio Herrera, Chile
Brian Koos, USA
Carina Mallard, Sweden
Donald Peebles, United Kingdom
Dan Rurak, Canada
Lucy Green, United Kingdom
Laura Bennet, New Zealand
Luc Zimmermann, The Netherlands
Bill Parer, USA
Tomoaki Ikeda, Japan
Tim Moss, Australia
Charles Wood, USA
Minutes of the FNPS Annual General Meeting

Grand Mercure Rockford Resort, Palm Cove, Queensland, Australia. Wednesday 13 July 2011.

Present: Laura Bennet, Dino Giussani (Scribe), Shinji Katsuragi (representing Tomoaki Ikeda), Tim Moss, Jan Nijhuis (Chair), Bill Parer, Donald Pebbles, Dan Rurak, Luc Zimmermann.

Meeting minutes. The minutes of the Beaulieu, Hampshire meeting held in the UK in 2010 were accepted with a few clarifications.

Jan Nijhuis expressed a vote of thanks to the organisers and sponsors.

There was a moment of silence to pay respect and reflect on the contributions of Mont Liggins and Bo Gennser.

FNPS Board membership. Emilio Herrera replaced Anibal Llanos. Emanuela Marinoni stepped down.

FNPS Archives. The possibility of indexing the FNPS volumes and abstracts at the University of Cambridge was discussed. Dino Giussani to investigate.

Future meetings:

2013. Chile. Organised by Emilio Herrera.

Prizes:
Tania Gunn Memorial Prize Postdoc Fellow - Tamara Yawno, The Ritchie Centre
Tania Gunn Memorial Prize PhD student - Miriam Nyberg, University of Bergen
Student Poster Prize - Stacey Ellery, The Ritchie Centre
Student Poster Prize - Carlie Cullen, University of Queensland.

Respectfully submitted,

Dino A. Giussani, PhD

FNPS Scribe
Advance Notice FNPS 2013 Chile

40th Annual Meeting of the Fetal and Neonatal Physiological Society

1-4 September 2013 - Chile

Come and enjoy FNPS 2013!

ORGANIZING COMMITTEE
Emilio Herrera, Claudia Torres-Farfan, Marcela Díaz, Germán Ebensperger

Universidad de Chile
Universidad Austral de Chile

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Chile
Sponsors

We are most grateful to the following for financial support
Welcome

It is our honour and pleasure to welcome you to the 39th meeting of the Fetal and Neonatal Physiological Society (FNPS). We are very pleased about the large number of abstracts submitted by so many young researchers from all over the world. This indicates that the FNPS is alive and well, and that FNPS meetings are highly appreciated by many, thanks to its informal character and lack of rigid structure.

The facilities at the venue, the estate of Woudschoten at Zeist, enable the exchange of scientific ideas and lively discussions between clinicians and basic scientists, but also offer great opportunities for pleasant socializing.

This year’s meeting will celebrate the career of Prof. Gerard Visser who retired from clinical work last year. He is now, more than ever, engaged in matters concerning perinatology at the international level. As a former fellow at the Nuffield Institute, Oxford UK, and as past president of the FNPS, he is the person designated to deliver the prestigious Dawes lecture and to share his stimulating, sometimes provocative, thoughts with us.

Furthermore, we will show you a glimpse of the city of Utrecht, known for its “broken” church, old university, and terraces along the canals (note that the film “Amsterdamned” was shot in Utrecht).

And then there is the breath-taking and traditionally infamous sporting event which will remain secret until the last moment.

Please take advantage of your time in The Netherlands and have an enjoyable meeting.

The FNPS 2012 Organising Committee.
Abstract Awards

This year, (PhD) students and “early” post-docs (<3 years from graduation) may win either the Tania Gunn Memorial Prize for best oral presentation or the Bo Gennser Memorial Prize for best poster presentation.

The Tania Gunn Memorial Prize was introduced last year in memory of the late 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.

The Bo Gennser Memorial Prize (information kindly provided by Karel Maršál and Tim Wheeler).

For the first time the best scientific poster presentation at the FNPS meeting will be awarded the Bo Gennser Memorial Prize. The prize was established thanks to the generous donation by Mrs Margit Gennser and her heirs to honour one of the founders and honorary members of the Fetal and Neonatal Physiological Society, Bo Gerhard Gennser (1929-2010).

Bo Gennser was born and grew up in Malmö, Sweden. He trained in obstetrics and gynecology at the University Hospital in Malmö, Lund University, where he worked for many years as consultant, senior lecturer and associate professor. In 1988, he moved to a similar position at the Karolinska Institute in Stockholm. Bo Gennser’s research focussed on fetal physiology. In 1972, he successfully defended his PhD thesis “Studies on myocardial function and carbohydrate metabolism in the foetus” and then closely collaborated with Professor Geoffrey Dawes at the Nuffield Institute in Oxford. Bo built up a perinatal research group at the University Department of Obstetrics and Gynecology in Malmö and was a very much appreciated mentor of many Swedish PhD students. Thanks to his fascination with modern technology and his close contacts with the Technical University in Lund, he and his group developed new methods for recording of fetal breathing movements and the evaluation of pulse waves in the fetal descending aorta based on 2-dimensional real-time ultrasound. In 1988, Bo Gennser published the first report on the association between low birth weight and risk of high blood pressure in adulthood in the BMJ.

In 1974, Bo Gennser and Geoffrey Dawes initiated the Fetal Breathing conferences that later developed into the Fetal and Neonatal Physiological Society. Bo was very active within our Society that was and still is a very important forum, especially for young scientists and clinicians interested in fetal physiology. Bo Gennser organized three of the annual meetings of the Society in Malmö, Sweden.

Bo was interested not only in fetal physiology, but in all fields of medicine and science, as well as in literature, the arts, and gardening. Thanks to his wide scope of interests and friendly attitude, the sometimes endless discussions were never boring and will be missed by the members of the Society.
Programme FNPS 2012

Sunday 8th July

13:00 Arrival at Woudschoten
13:00 – 14:30 Conference registration; Accommodation check-in; Posters to be put on display
14:30 – 14:45 Meeting open and Welcome

14:45 – 16:30 **Session I – Cardiovascular, part A**

*Chairs: Dino Giussani & Gerard Visser*

O1. Post-asphyxial hypoperfusion: a role for neural, but not endothelial control?
   *Bennet L, Quaedackers JSLT, Booth LC, Gunn AJ*

O2. Postnatal oxygenation at sea level modifies the remodeling and reactivity of the small pulmonary artery in neonatal lambs born in the Andean Altiplano.
   *Ebansperger G, Herrera EA, Montt C, Hernández I, Riquelme AN, Moraga FA, Reyes RV, Llanos AJ*

O3. Regulation of microvascular blood flow by heme oxygenase in the preterm neonate.
   *Shepherd K, Palliser H, Dyson R, Wright I*

   *Dyson R, Palliser H, Latter J, Chwatko G, Glowacki R, Wright I*

O5. Antioxidants prevent growth restriction and cardiac dysfunction in chronically-hypoxic fetal sheep.
   *Brain K, Allison B, Niu Y, Cross C, Itani N, Herrera EA, Giussani DA*

O6. B-type natriuretic peptide after (chronic) fetal hypoxia.
   *Vijlbrief D, Benders M, Kemperman H, Pistorius L, van Bel F, de Vries W*


16:30 – 17:00 Coffee break & poster viewing

17:00 – 18:30 **Session II – Poster session with blitz oral presentations (see page 22)**

*Chairs: Jan Derks, Joepe Kaandorp & Deodata Tijsseling*

18:30 – 19:00 The **Geoffrey Dawes Lecture** by Gerard Visser (Prof. of Obstetrics, UMC Utrecht).
   *Title: “An exciting time for obstetricians”.*
Monday 9th July

07:00  Breakfast

08:00 – 09:45  Session III  –  Growth & Nutrition  
   *Chairs: Donald Peebles & Karel Maršál*

O8.  Local administration of Ad.VEGF-A165 to the utero-placental circulation enhances fetal growth and attenuates brain sparing in a fetal growth restriction model of guinea pig pregnancy.  
*Mehta V, Boyd M, Barker H, Avdic-Belltheus A, Carr D, Martin J, Zachary I, Peebles D, David AL*

O9.  Is placental nutrient transport affected by pregnancy rank in sheep?  
*van der Linden D, McCoard S*

O10.  Maternal insulin sensitivity in mid pregnancy does not determine birth weight after embryo transfer between large and small sheep breeds.  

O11.  The origin of fetal sterols in second trimester amniotic fluid: endogenous synthesis or maternal-fetal transport?  
*Baardman M, Erwich JJ, Berger R, Hofstra R, Kerstjens-Frederikse M, Lütjohann D, Plösch T*

O12.  The effect of grand maternal nicotine exposure during gestation and lactation on lung integrity of the F2 generation.  
*Maritz GS, Mutemwa M*

O13.  Thyroid hormone deficiency alters insulin signalling proteins in skeletal muscle of fetal sheep near term.  
*Forhead AJ, Jellyman JK, Martin-Gronert MS, Ozanne SE, Shen QW, Du M, Fowden AL*

O14.  Real-time ultrasound assessment of body and breathing movements and abdominal diameter in fetal lambs from 55 d gestation to term.  
*Rurak D, Wittmann B*

09:45 – 10:15  Coffee break & poster viewing
10:15 – 12:00 **Session IV – Brain, part A**  
*Chairs: Dan Rurak & Manon Benders*

**O15.** Perinatal hypoxia impairs synaptic plasticity in the hippocampus of male but not female marmosets later in life.  
*Coleman HA, Walker DW, Wong F, Parkington HC*

**O16.** Pre-existing hypoxia in multiple pregnancies predisposes to systemic compromise, impaired cerebral perfusion, and greater EEG suppression during repeated brief umbilical cord occlusions in near-term fetal sheep.  
*Wassink G, Benet L, Westgate J, Gunn AJ*

**O17.** Hypothalamic global gene expression in response to hypoxia or brachiocephalic occlusion in late-gestation fetal sheep.  
*Wood CE, Chang E, Richards E, Rabaglino MB*

**O18.** Prenatal hypoxia and neurological disease in adulthood: role of oxidative stress.  
*Camm E, Lusby C, Tijsseling D, Kane A, Cross C, Derks JB, Giussani DA*

**O19.** Connexin 43 hemichannel blockade: mimetic peptide dose response after ischaemia.  
*Davidson JO, Green CR, Benet L, Nicholson LFB, Gunn AJ*

**O20.** Insulin-like growth factor (IGF)-1 increases gliosis but reduces caspase-3 activation in grey matter after ischemia in near-term fetal sheep.  
*Mathai S, Benet L, George SA, Lenders D, Gunn AJ*

**O21.** Exposure to maternal glucocorticoids exacerbates post-asphyxial brain injury in the preterm fetus.  
*Koome M, Drury PP, Davidson JO, Mathai S, George SA, Gunn AJ, Benet L*

12:00 – 12:30 Guest Lecture by Cobi Heijnen (Prof. of Psychoneuroimmunology, UMC Utrecht).  
*Title: “Neuroregeneration by mesenchymal stem cells after neonatal brain injury”.*

12:30 – 13:30 Lunch

13:30 – 15:00 **Session V – Miscellaneous**  
*Chairs: Keiji Suzuki & Luc Zimmermann*

**O22.** Ovine fetal thymus response to lipopolysaccharide-induced chorioamnionitis and antenatal corticosteroids.  
*Kuypers E, Collins JJP, Jellema RK, Wolfs TGAM, Kemp MW, Nitsos I, Pillow JJ, Polglase GR, Newnham JP, Germeraad WTV, Kallapur SG, Jobe AH, Kramer BW*

O24. Hypoxia development in patients with transposition of the great arteries according to timing of arterial switch operation. *Fedevych O, Chasovskyi K, Vorobyova H, Zhovnir V, Yemets I*


O27. Effect of SSRI exposure on fetal general movements, case-control study. *van Lunteren EF, Blijleven S, de Vries JIP, Visser GHA, Ververs FFT, Mulder EJH*

15:00 – 15:15 Society Board Announcements & Advance Notice FNPS 2013, Chile

15:15 – 15:45 Coffee break & poster viewing *sponsored by ACE Pharmaceuticals BV*

15:45 – 17:15 Session VI – Stress
*Chairs: Bea Van den Bergh & Eduard Mulder*

O28. Prenatal maternal psychosomatic stress: effects on fetal brain development following maternal neurosteroid treatment in guinea pigs. *Bennett GA, Palliser HK, Kelleher MA, Saxby BM, Walker DW, Hirst JJ*


O32. Parents’ depressive symptoms during pregnancy and postpartum: effects on toddler’s behavior problems. *Canário C, Figueiredo B*
O33. Cortisol independent transfer of maternal stress effects to the fetus.

17:15 – 18:15 Drinks
18:30 Coaches depart for trip to dinner site in Central Utrecht
19:00 Society Dinner at Academy Hall, Utrecht University
23:00 Coaches leave Utrecht City for Woudschoten
>23:00 Optional pub crawl

**Tuesday 10th July**

07:00 Breakfast

08:00 – 10:00 *Session VII – Preterm birth, Lungs & Placenta*
   *Chairs: Alison Forhead & Graham Jenkin*

O34. The guinea pig model of human parturition.
   Zakar T, Hirst J, Welsh T

O35. Human labour is associated with decreased myometrial ether-a-go-go related gene (hERG) potassium channels that modulate contractility.
   Parkington HC, Paul J, Tonta MA, Chan E-C, Sheehan PJ, Brennecke SP, Coleman HA, Smith R

O36. Placental mitochondrial uncoupling protein 2 and perinatal inflammation: exposing the preterm infant to increased reactive oxygen species?
   Hodyl NA, Aboustate N, Clifton VL, Stark MJ

O37. Effect of antenatal corticosteroids on the TGFβ-pathway and Caveolin-1 in the ovine fetal lung after LPS exposure.
   Collins JJP, Kunzmann S, Kuypers E, Kemp MW, Newnham JP, Kallapur SG, Jobe AH, Kramer BW

O38. Fetal leptin administration during late gestation improves aspects of lung function and maturation in the fetal sheep.
   De Blasio MJ, Kempster SL, Smith GCS, Wooding FBP, Blache D, Fowden AL, Forhead AJ

O39. Statins in the newborn period mature the lungs.
   Allison B, Bronckers I, Murphy D, Camm E, Cross C, Kane A, Niu Y, Herrera E, Lotgering F, Giussani DA

O40. Statins prevent detrimental effects of postnatal glucocorticoid therapy on arterial blood pressure and the kidney in rats.
   Itani N, Kane A, Cross C, Camm E, Niu Y, Herrera E, Giussani DA
O41. Differential oxidative stress responses in preterm and term placenta following n-3 fatty acid administration. 
   Hodyl NA, Clifton VL, Stark MJ

10:00 – 10:30 Coffee break & poster viewing  
   sponsored by Nemo Healthcare

10:30 – 12:30 Session VIII – Brain, part B  
   Chairs: Laura Bennet & Frank van Bel

O42. Defining the risk of early brain injury or death in the very preterm newborn: Measurement of cerebral oxygen extraction during the first 24 hours of life.  
   Stark M, Balegar KK, Andersen C

O43. Effect of hypothermia on periventricular leukomalacia via attenuating the cell death of oligodendrocyte precursor cells.  
   Ichinose M, Kamei Y, Imada S, Seyama T, Iriyama T, Kozuma S, Taketani Y, Aso H

O44. Differences in vulnerability of the developing and juvenile rat brain to TBTO demonstrated with structural MRI and functional [18F]FDG brain microPET imaging.  

O45. Early glutamate receptor blockade does not augment hypothermic neuroprotection of the striatum in preterm fetal sheep.  
   George SA, Barrett R, Bennet L, Jensen E, Mathai S, Gunn AJ

O46. Postnatal melatonin improves cerebrovascular function in neonates gestated under chronic hypoxia.  
   Herrera E, Montt C, Ebensperger G, Santos D, Diaz M, Chubretovic M, Reyes R, Serón-Ferré M, Llanos A

O47. Statins prevent adverse effects of postnatal glucocorticoid therapy on the developing brain in rats.  

O48. Melatonin as a potential neuroprotective therapy in intrauterine growth restriction.  

O49. Melatonin protects the growth restricted fetal brain following glucocorticoid administration.  
   Sutherland AE, Bennet L, Yawno T, Jenkin G, Wallace EM, Miller SL
12:30 – 13:00  Guest Lecture by Manon Benders (Assoc. Prof. of Neonatology, UMC Utrecht).
   Title: “MRI and brain development”.

13:00 – 14:00  Lunch

14:00  Coaches depart for sporting event & conference diner
22:30  Bar open at the venue

Wednesday 11th July

07:00  Breakfast

08:00 – 10:00  **Session IX – DOHaD**
   *Chairs: Emilio Herrera & Claudia Torres-Farfan*

O50.  Low dose prenatal alcohol exposure induces an increase in anxiety-related behaviour but does not effect pyramidal cell number in the basolateral amygdala.
   *Cullen C, Lavidis N, Burne T, Moritz K*

O51.  Maternal melatonin suppression imposed by gestation under constant light has pronounced effects on global gene expression in the rat fetal heart and liver.
   *Spichiger C, Galdames H, Abarzua-Catalan L, Mendez N, Alonso P, Gutierrez S, Torres-Farfan C, Richter H*

O52.  Gender effects on renal ageing.
   *Clifford B, Pijacka W, Joles JA, Langley-Evans S, McMullen S*

O53.  Adrenocortical remodeling following prenatal dexamethasone treatment in a novel species – the spiny mouse.
   *Quinn T, Dickinson H, Walker DW*

O54.  Impact of sex steroids on renal AT2R expression and the progression of ageing related renal injury.
   *Pijacka W, Joles JA, Tilburgs C, Clifford B, Langley-Evans S, McMullen S*

O55.  The rat hippocampus circadian clock: Developmental programming of spatial memory deficit by materno/fetal melatonin suppression secondary to chronodisruption.
   *Vilches N, Abarzua-Catalan L, Mendez N, Spichiger C, Galdames H, Hazlerigg DG, Richter HG, Torres-Farfan C*

O56.  Postnatal β-adrenergic desensitization caused by chronic prenatal hypoxia is linked to a decreased β1/β2AR ratio and an increase in Gαs.
   *Lindgren I, Altimiras J*
O57. Does concurrent growth restriction alter the effects of neonatal hyperoxia on pulmonary structure and function in adulthood?  
*Sozo F, O’Reilly M, Hansbro P, Horvat J, Beckett E, Harding R*

10:00 – 10:30 Coffee break

10:30 – 11:15 Keynote Lecture by Dino Giussani (Prof. of Developmental Cardiovascular Physiology & Medicine, Cambridge, UK).  
*Title:* “Heart Disease Link to Oxygen in the Womb: An Evolutionary Perspective”.

11:15 – 12:45 **Session X – Cardiovascular, part B**  
*Chairs:* Helena Parkington & Willem de Vries

O58. Intra-amniotic lipopolysaccharide exposure induces aberrations in fetal heart development.  
*Bensley JG, Polglase GR, Moss TJM, Gill AW, Kluckow M, De Matteo R, Harding R, Black MJ*

O59. Smoking in pregnancy: Effects on fetal heart rate variability.  
*Kapaya H, Broughton Pipkin F, Loughna P, Hayes-Gill BR*

O60. Heart and coronary vessel function in normally grown and growth restricted sheep fetuses treated with melatonin.  
*Parkington HC, Tare M, Sutherland AE, Yawno T, Jenkin G, Coleman HA, Wallace EM, Miller SL*

O61. Increased activated caspase-3 in conductive cells in the fetal heart after prolonged increase in maternal cortisol.  
*Keller-Wood M, Feng X*

*Wright I, Latter J, Buchan J*

O63. Postnatal melatonin modifies the cardiopulmonary function in high altitude newborn sheep.  

12:45 Prize-winning Abstracts & Meeting closure

13:00 Lunch

14:00 Departure
Poster Presentations

Sunday 8th July  Session II
Chaired by Jan Derks, Joepe Kaandorp & Deodata Tijsseling

Brain

P01. Connexin 43 hemichannel blockade is not beneficial during ischaemia in the near-term fetal sheep. 
Davidson JO, Green CR, Bennet L, Nicholson LFB, Gunn AJ

P02. Endotoxin-mediated sensitization of the neonatal brain prior to hypoxic-ischemic insult: the role of the TNF family of pro-inflammatory cytokines TNFα, LTα and LTβ. 
Rocha-Ferreira E, Sidhu B, Thei L, Rahim A, Lange S, Hristova M, Raivich G

P03. C-Jun plays a regulatory role in the neonatal brains response to hypoxia-ischemia induced injury. 
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Session I – Cardiovascular, part A

Chaired by Dino Giussani & Gerard Visser
Post-asphyxial hypoperfusion: a role for neural, but not endothelial control?

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Introduction: Hypotension and hypoperfusion is observed in many preterm infants, particularly in the early hours of life, and are believed to initiate or exacerbate injury. Most current management is based on the concept that if organ blood flow is poor, then it must be related to inadequate perfusion pressure. In practice, recent studies suggest that for most infants, particularly in the first few days of life, changes in vascular resistance rather than blood pressure are the primary factor determining blood flow. This may occur during recovery from in utero hypoxia, but the factors mediating increased vascular resistance and thus treatment options remain unclear. The aim of this preliminary study was to examine the role of neural and endothelial factors in mediating fetal hypoperfusion after asphyxia.

Methods: Fetal sheep at 0.7 gestation underwent asphyxia induced by 25 minutes of complete umbilical cord occlusion. Post-occlusion, fetuses received 8 h infusions of the alpha-adrenergic antagonist phentolamine started at 15 or 30 min post-occlusion, and the nitric oxide donor L-arginine or the endothelin antagonist bosentan started 15 min post-occlusion. Renal sympathetic nerve activity (RSNA) was monitored by telemetry in a subset of fetuses.

Results: Phentolamine infusion started at 15 min, but not 30 min, after the end of occlusion prevented the post-asphyxial hypoperfusion. This effect was sustained after the end of infusion. Neither L-arginine nor tezosentan prevented hypoperfusion, despite repeated top-up boluses. RSNA was significantly increased during post-asphyxial hypoperfusion, with maximal nerve activity observed at the nadir of blood flow.

Conclusion: Hypoperfusion post-asphyxia is not mediated by altered endothelial function, but rather there is sympathetic nervous system activation, likely facilitating coupling of blood flow to reduced metabolism. However, failure to reverse hypoperfusion with delayed phentolamine suggests an early loss of alpha-adrenergic regulation of hypoperfusion, or altered alpha-adrenergic sensitivity.
Postnatal oxygenation at sea level modifies the remodeling and reactivity of the small pulmonary artery in neonatal lambs born in the Andean Altiplano

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Gestations submitted to chronic hypoxia from high altitude yields neonates with pulmonary arterial hypertension. This is due to an increased vascular tone and vascular remodeling. Several vasoactive mechanisms are changed in high-altitude hypoxia modifying the vascular responses to this environment. However, it is not known whether the vascular structure and reactivity changes when a high altitude newborn is submitted in the first days after birth to the high O₂ milieu found at sea level. We hypothesize that postnatal oxygenation changes the vascular structure and vasoactive function in the small arteries of the pulmonary circulation. Nine newborn (NB) lambs fully gestated and born in Putre (3,600m) were divided in two groups, five NB were kept in Putre (HAHA) and 4 NB were brought down to Lluta (HALA, 60m) at 2-3 days after birth. The lambs underwent euthanasia at 14-16 days and the right lung was removed by dissection and immediately immersed in cold saline. Fourth-fifth order pulmonary arteries were dissected. Isolated arteries were mounted in a wire myograph and maintained at 37°C aerated with 95% O₂-5% CO₂. Concentration-response curves (CRCs) were made for KCl, endothelin-1, U46619 (thromboxane A2 analog) sildenafil (PDE-5 inhibitor), NS 1619 (activator of BKCa channels), 8-Br cGMP (activator of PKG-1) and sodium nitroprusside (NO donor). The HALA NB lambs had a decreased vasodilation to NO, PKG and BKCa in relation to HAHA group. There was also a decreased vasoconstriction to KCl, ET-1, U46619 and PDE5 in HALA vs. HAHA lambs (p<0.05). Furthermore, postnatal oxygenation produced in the small pulmonary arteries a decrease in the smooth muscle area. In conclusion, postnatal oxygenation at sea level markedly reduced the pulmonary arterial thickness, vasodilator and vasoconstrictor responses in the small pulmonary arteries in neonates brought down from the Alto Andino, after 15 days at sea level.

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O3

Regulation of microvascular blood flow by heme oxygenase in the preterm neonate

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Introduction: In the preterm neonate, high blood flow through the microvasculature is associated with physiological instability and poor outcome in the first 72 hours of life. Preterm males have increased risk of poor outcome and higher levels of baseline microvascular flow. Carbon monoxide (CO) has been associated with this microvascular dilatation. Endogenous CO is produced by enzymatic degradation of heme by two heme oxygenases; 1 (HO-1) and 2 (HO-2), eliciting relaxation of vascular smooth muscle. We aimed to determine HO expression in our model of prematurity and microvascular dysfunction. We hypothesised that expression of HO would be altered by gestational age (GA) and sex.

Materials and Methods: Preterm (GA62±1) and term (GA69±1) guinea pigs were delivered by caesarean section. At 23 hours postnatal age (PNA), laser Doppler flowmetry was used to study microvascular behaviour. Fetal (0 hour; preterm n=12; term n=10) and neonatal (24 hour; preterm n=12; term n=12) tissues were collected. Skin samples (external ear) were collected at post mortem and HO-1 and HO-2 protein levels were quantified by western blot analyses.

Results and Discussion: At 23hr PNA, preterm animals exhibited significantly higher microvascular blood flow compared to term (p=0.03), suggesting increased microvascular dilatation. An effect of GA was observed on HO-1 expression in male fetal animals with preterm animals having an increased expression (p=0.05). Overall male neonates exhibited a positive correlation between HO-1 and HO-2 expression (r=0.61, p=0.04). Interestingly, a significant effect of PNA was observed only in male animals, with HO-1 expression increasing with PNA (p=0.003).

Conclusion: Significant relationships between HO-1 expression and male sex suggest physiological role in both 0hr and 24hr neonates. No direct correlations are evident in males between HO-1, HO-2 expression and baseline microvascular blood flow suggesting dysregulation of the HO/CO-pathway at this time point may not be the predominant mechanism underlying microvascular dysfunction, warranting further investigation of alternate pathways.
Hydrogen sulphide in the neonatal transitional circulation

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Introduction: Regulation of microvascular tone during the perinatal period is associated with physiological instability and illness severity. High microvascular blood flow in the preterm neonate has been linked to poor outcome. Little evidence of the mechanisms controlling microvascular tone during circulatory transition exist, however previous studies suggest a potential role of the gasotransmitters. This study aimed to assess hydrogen sulphide (H\textsubscript{2}S) levels in newborns and to establish if any relationship exists between H\textsubscript{2}S and microvascular function.

Materials and Methods: Term (≥37wk GA), preterm (29≤36wk GA) and very preterm (24≤28wk GA) neonates were studied. Microvascular blood flow was assessed by laser Doppler flowmetry. 24hr pooled urine samples from the first 3 days of life were collected. Thiosulphate, a urinary metabolite of H\textsubscript{2}S, was determined by high performance liquid chromatography. Thiosulphate was standardised to 24hr urinary output and birth weight as a measure of total body H\textsubscript{2}S turnover.

Results and Discussion: H\textsubscript{2}S turnover was stable across the first 72hrs of life in term neonates. In very preterm neonates, H\textsubscript{2}S turnover increased significantly from day 1 to 3 (p=0.005). A significant interaction of gestational age and sex was observed for total body turnover of H\textsubscript{2}S. In the 24–28wk group, male infants had higher H\textsubscript{2}S turnover than females (p=0.039). Interestingly, no correlation between microvascular blood flow and H\textsubscript{2}S turnover was observed in these infants. A significant relationship between microvascular blood flow and H\textsubscript{2}S turnover was observed on day 2 of postnatal life in the 29-36wk group (p=0.008).

Conclusion: Neonates at the greatest risk of microvascular dysfunction characterised by inappropriate peripheral vasodilatation - very preterm male neonates - are also the neonates with highest levels of total body H\textsubscript{2}S turnover. The results in more mature preterm infants suggest a role in physiologic microvascular tone regulation for H\textsubscript{2}S as a vasodilator during circulatory transition.
Antioxidants prevent growth restriction and cardiac dysfunction in chronically-hypoxic fetal sheep

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Introduction: Intrauterine growth restriction (IUGR) is associated with infant death and with cardiovascular disease in adulthood1. There is no cure for it. One of the most common causes of IUGR is chronic fetal hypoxia, which is also known to programme cardiovascular dysfunction2. However, the mechanisms underlying these associations remain unclear. The sheep fetus has long been the model of choice for investigating fetal hypoxia. It is accepted that the sheep fetus can adapt successfully to acute hypoxia3. However, the effects of chronic fetal hypoxia in ovine pregnancy have been restricted to a few seminal studies4-9. We have now developed 4 isobaric hypoxic chambers able to maintain pregnant sheep for the duration of gestation. In this study, we tested the hypothesis that IUGR and developmental origins of cardiac dysfunction in chronic hypoxic pregnancy are secondary to oxidative stress in the fetal cardiovascular system.

Methods: Chronically catheterised sheep carrying male singletons were exposed to normoxia or hypoxia (10% inspired O2) ± vitamin C (maternal 200mg.kg\(^{-1}\) i.v. daily) for the last third of gestation (105-138 days; term~145 days). At 138 days, fetuses were delivered and weighed, their tissues collected and cardiac function was investigated in a Langendorff preparation.

Results: Maternal P\(_{a}\)O\(_2\) and maternal haemoglobin [Hb] were similarly altered during chronic hypoxia (\textit{Mat P\(_{a}\)O\(_2\)}: N=105.8±1.8; H=47.3±0.6; HC=47.5±0.7; NC=107.1±2.3 mmHg; \textit{Mat [Hb]}: N=10.0±0.2; H=12.1±0.1; HC=12.1±0.1; NC=10.0±0.2 g.dl\(^{-1}\); all P<0.05, N vs. H). Relative to controls, growth was reduced, left ventricular end diastolic pressure was increased and myocardial contractility and relaxability were reduced in chronically-hypoxic fetuses. Treatment of hypoxic pregnancies with vitamin C prevented these effects (Fig. 1).

Conclusions: Maternal treatment with antioxidants prevents IUGR and fetal systolic and diastolic dysfunction in chronically hypoxic pregnancy. This study provides insight to mechanism and targets for clinical intervention in risky pregnancy.
**Figures:** Values are mean ± S.E.M. N, normoxic; H, hypoxic; HC, hypoxic with vitamin C; NC, normoxic with vitamin C. Significant differences are: *P<0.05, (One-Way ANOVA with Tukey Test). LVEDP, left ventricular developed pressure; dP/dt_{max} represents myocardial contractility.

**Supported by The British Heart Foundation and the BBSRC.**

**References:**

O6

B-type natriuretic peptide after (chronic) fetal hypoxia

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Introduction: Impairment of gas exchange and blood flow through the placenta leads to hypoxia and hypercapnia, causing increased systemic vascular resistance and tachycardia, thus compromising the cardiovascular system of the foetus. The biomarker B-type natriuretic peptide (BNP) can be used to identify significant cardiovascular compromise in infants. The objective was to investigate whether BNP can be used to identify those preterm infants with significant cardiovascular compromise during peripartum period.

Materials and Methods: In this retrospective cohort study all infants born after a gestational age of less than 32 weeks were evaluated. Factors associated with prenatal and perinatal hypoxia-ischemia were related to BNP levels after birth. Pathologic examination of the placenta was routinely performed.

Results and Discussion: In total 164 infants were evaluated. BNP was found to be associated with (chronic) prenatal hypoxia-ischemia (nucleated red blood cells (r² 0.22; p<0.001); intrauterine growth retardation (r² 0.18; p<0.01); postnatal thrombocytopenia), and acute perinatal hypoxia (umbilical artery pH (r² 0.14; p<0.001); serum lactate (r² 0.11; p<0.001); 1 and 5 minute Apgar scores). Furthermore an association was found between signs of placental ischemia on pathologic examination and elevated BNP levels.

Conclusion: Elevated BNP levels after birth are found in those preterm infants with significant pre and perinatal hypoxia-ischemia and can possibly be used to identify those infants at risk for cardiovascular compromise.
Maternal outcome in pregnancy complicated with pulmonary hypertension

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Background: Pulmonary arterial hypertension (PAH) including Eisenmenger syndrome has a risk of mortality in pregnancy of 10-40%. The aim of the study was to investigate whether pulmonary artery blood pressure (PABP) is a prognostic factor for pregnancy outcome in patients with PAH.

Methods and Results: The subjects were 42 patients with PAH during pregnancy. Severe and mild cases were defined by PABP before and during the first 14 weeks of pregnancy, with severe cases having mean PABP >40 mmHg by catheterization or systolic PABP >50 mmHg on echocardiography. Eighteen women chose termination of pregnancy before 14 weeks, leaving 24 women (10 mild, 14 severe) for analysis. The severe cases delivered earlier (35.4 vs. 31.5 weeks, P<0.05) and had higher rates of small-for-gestational-age infants (0/10 vs. 7/14, P<0.01). In severe cases, the NYHA class dropped by 1 in 9 cases, by 2 in 3 cases, and remained the same in 2 cases as pregnancy progressed, whereas 1 mild case dropped 1 class and 9 remained the same. In severe cases, there was one maternal death and one fetal death. PABP was markedly elevated in later pregnancy from 54 to 74 mmHg (catheter measurement) and 78 to 93 mmHg (echocardiography) in severe cases (P<0.05).

Conclusion: PABP before or in the early stage of pregnancy is an important predictor of pregnancy outcome.
Session III – Growth & Nutrition

Chaired by Donald Peebles & Karel Maršál
Local administration of Ad.VEGF-A$_{165}$ to the utero-placental circulation enhances fetal growth and attenuates brain sparing in a fetal growth restriction model of guinea pig pregnancy

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Introduction: Fetal growth restriction (FGR) is commonly caused by impaired utero-placental perfusion limiting fetal nutrient and oxygen supply. We have shown that adenovirus (Ad) VEGF-A$_{165}$ expression in the uterine arteries (UtAs) of mid-gestation pregnant sheep significantly increases UtA blood flow long term, compared with UtAs transduced with a control adenovirus encoding β-galactosidase (Ad.LacZ)$^1$. Recent work in the adolescent overnourished ewe paradigm of FGR shows that UtA injection of Ad.VEGF-A$_{165}$ increases fetal growth velocity$^2$. We have now investigated the effect of Ad.VEGF-A$_{165}$ on fetal growth in the FGR guinea pig.

Materials and Methods: To create FGR, virgin Dunkin-Hartley guinea pigs were nutrient restricted peri-conceptually. Under general anaesthesia at mid-gestation (30-34 days of gestation), sonographic fetal measurements were recorded in nutrient restricted pregnant sows and control ad lib fed sows. In FGR guinea pigs and at laparotomy, the UtAs were dissected free of fat, and the UtAs and radial arteries on each side were transduced externally with 5x10$^9$ viral particles of Ad.VEGF-A$_{165}$ or Ad.LacZ, combined with a thermosensitive pluronic gel. The guinea pigs were sacrificed 31-34 days post-surgery but before birth. Fetal organ weights and biometry were recorded.

Results and Discussion: There was no maternal or fetal morbidity and mortality. Nutrient restriction reduced fetal weight by 40% reduction and increased brain to liver weight ratio (brain sparing). In FGR pregnancies, administration of Ad.VEGF-A$_{165}$ increased fetal weight (94.5±2.01g, n=11) when compared to control Ad.LacZ treated fetuses (84.9±2.81g, n=10, p=0.061). The liver and kidney weights were significantly higher in the Ad.VEGF-A$_{165}$ group (5.6±0.23g v/s 4.7±0.18g, p=0.019 and 0.74±0.065g v/s 0.37±0.021g, p<0.001 respectively), and the brain/liver weight ratio was significantly lower (0.45 ± 0.019 v/s 0.53 ± 0.017, p=0.021), suggesting an attenuated brain sparing effect.

Conclusion: Ad.VEGF-A$_{165}$ transduction of the utero-placental vasculature enhances fetal growth and reduces brain sparing in nutrient restricted fetal guinea pigs.

References
Is placental nutrient transport affected by pregnancy rank in sheep?

Danitsja van der Linden and Sue McCoard

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Understanding the link between placental function and fetal growth is critical in order to comprehend the mechanisms underlying altered fetal growth. In humans, the placenta has been found to be the link between amino acids, the insulin-IGF axis and low birth weight, however, it is unknown if a similar link exists in sheep. This study investigated the insulin, IGF-I and free amino acid (AA) profiles in fetal, umbilical artery and vein plasma of singleton and twin fetuses in late pregnancy. These profiles were used as an indicator of placental nutrient transport. Singleton and twin placentae, from ewes offered ad libitum grazing throughout pregnancy were studied at day 140 of pregnancy. Blood samples from each fetus, umbilical vein and artery were collected. Individual placentae per fetus were dissected and placentome number and weight were recorded. Twin fetuses were 16% lighter (P=0.01) than singletons, and had a smaller placenta with 28% decreased placentome weight (P=0.03) and 35% fewer placentomes (P=0.001). In twins, umbilical artery plasma had 55% lower Asn (P=0.003) and 45% higher Glu (P=0.005) concentrations than fetal plasma, but no differences between these pools were observed in singletons. Glutamate is a major oxidation-energy source for the placenta and the fetal liver is the net producer of Glu. This may indicate that the functionality of the feto-placental unit is different between singletons and twins. Overall, twin fetuses had 29% lower insulin (P=0.048), 35% lower IGF-I (P=0.001), 12% lower His (P=0.095), 14% lower Leu (P=0.085) and 29% lower Arg (P=0.133) concentrations than singletons. Arginine, His, and Leu are examples of AA that can promote insulin secretion and, in turn, insulin can increase fetal IGF-I concentrations. Insulin and IGF-I are important fetal growth factors by stimulating and regulating AA transport across the placenta. Collectively, these results indicate that AA transport may be reduced in twin placentae or that the feto-placental unit of twins exhibit a different AA metabolism compared to singletons.
Maternal insulin sensitivity in mid pregnancy does not determine birth weight after embryo transfer between large and small sheep breeds

Mark Oliver\textsuperscript{1,3}, Anne Jaquiery\textsuperscript{1,3}, Paul Kenyon\textsuperscript{2,3}, Sarah Pain\textsuperscript{2,3}, Catriona Jenkinson\textsuperscript{2,3}, Hugh Blair\textsuperscript{2,3}, Maggie Honeyfield-Ross\textsuperscript{2,3}, and Frank Bloomfield\textsuperscript{2,3}

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\textsuperscript{2} Sheep Research Centre, Institute of Veterinary, Animal and Biomedical Sciences, Massey University.
\textsuperscript{3} National Research Centre for Growth and Development.

Introduction: Embryo transfer (ET) of large sheep breed embryos (S, Suffolk) into small breed ewes (C, Cheviot) leads to constrained birth size. Changes in maternal insulin sensitivity in pregnancy promote fetal substrate supply. We sought to examine what influence reciprocal ET crosses had on pregnancy related development of maternal insulin resistance in mid gestation.

Methods: Embryos were collected 6d post-mating from donors and transferred to recipients. Between and within breed transfers were performed: CinC, CinS, SinS & SinC. At 60-70d of pregnancy overnight fasted ewes underwent hyperinsulinaemic-euglycaemic clamps (HEC). Insulin sensitivity (InS) was determined when steady state (SS) glucose and insulin CVs were <10% and <20% respectively.

Results and Discussion: At mid gestation, plasma SS for glucose and insulin, and InS, were not different between ewes (Table). Ewe weight at HEC and required glucose infusion rate was higher in Suffolk than Cheviot ewes (both \(p<0.01\)). SinS lambs were heavier than other lambs at birth (\(p<0.05\)). Mid gestation InS does not appear to be a major factor in constraining the growth of a large breed sheep fetus transferred into smaller breed, although the trend towards higher InS in SinC than in CinC (\(p=0.1\)) suggests that it may play some role. It is possible InS during later pregnancy may be affected. However, as embryo size was already different between groups by day 19, this suggests factors other than maternal gestational insulin resistance determine fetal growth in this ET paradigm.

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<tr>
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<th>CinC(7)</th>
<th>CinS(9)</th>
<th>SinC(9)</th>
<th>SinS(7)</th>
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<td>Ewe weight at HEC (kg)</td>
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<td>SS glucose (mM)</td>
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<td>SS insulin (ng/ml)</td>
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<td>InS(mM/ml.ng/ml.kg/min)</td>
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<td>Lamb birth weight (kg)</td>
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Data are means±SEM. Numbers per group in brackets. Different letter postscripts indicate significance (\(p<0.05\)).
O11

The origin of fetal sterols in second trimester amniotic fluid: endogenous synthesis or maternal-fetal transport?

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Introduction: Cholesterol is crucial for embryonic development. We and others have shown that maternal cholesterol contributes substantially to the fetal cholesterol pool in animal models. To gain more insight into the origin of the fetal cholesterol pool in early human pregnancy we determined cholesterol levels and its precursors in the amniotic fluid of uncomplicated, singleton human pregnancies. We hypothesized that during early life, i.e. before the 19th week of gestation, the fetus is highly dependent on maternal cholesterol supply because the endogenous cholesterol synthesis by fetal tissues is relatively low.

Materials and Methods: Total cholesterol, lanosterol, dihydrolanosterol, lathosterol and desmosterol concentrations were analyzed as markers for fetal cholesterol synthesis in the second trimester amniotic fluid of 126 healthy fetuses from week 15 till week 22. To analyze maternal fetal cholesterol transport we measured β-sitosterol levels.

Results and Discussion: Lanosterol, dihydrolanosterol and lathosterol were present in very low levels until the 19th week of gestation, after which their levels increased very strongly (p < 0.001). Significant amounts of β-sitosterol were detectable in the amniotic fluid although these varied throughout the second trimester of pregnancy; desmosterol concentrations also varied. Total cholesterol levels increased slightly between weeks 15 and 22.

Conclusion: Our results support our hypothesis that during early life, i.e. before the 19th week of gestation, the fetus is highly dependent on the maternal cholesterol supply because the endogenous cholesterol synthesis by fetal tissues is relatively low. We show that maternally-derived sterols can cross the placenta and are detectable in the amniotic fluid. Therefore, the availability of maternal cholesterol early in embryogenesis might be an important factor for fetal development.
The effect of grand maternal nicotine exposure during gestation and lactation on lung integrity of the F2 generation

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Introduction: Maternal nicotine exposure during gestation and lactation adversely affects lung development in the offspring. It has been suggested that the "program" that control long-term maintenance of the structural integrity of the lung may be compromised. The aim of the study was to establish whether the effect of grand-maternal nicotine exposure during gestation and lactation can be transferred to the F2 generation.

Materials and methods: After mating, rats were randomly divided into two groups (F0). One group received nicotine (1 mg/kg body weight/day). The controls receive saline. Morphological and morphometric techniques [Body weight (BW), lung volume (Lv), linear intercept (Lm), alveolar wall thickness (Tsept), senescent and proliferating cell numbers] were used to evaluate changes in the lung structure of the offspring (F1) at postnatal days 21, 42, 63, and 84. The F1 generation was divided into 4 groups. Mating in the individual groups was as follows: 1) NmCf (F1 nicotine exposed male mated with F1 control female), 2) NfCm (F1 nicotine exposed female mated with F1 control male) and 3) NmNf (F1 male exposed to nicotine mated with F1 female also exposed to nicotine). The F1 nicotine exposed males and females were exposed to nicotine via the placenta and mother’s milk (F0 generation) only.

Results and Discussion: The effect of grand-maternal nicotine (F0) exposure is transferred to the F1 and F2 generations. This includes an increase in Lm, and thinner alveolar walls. Lv and BW were not affected. Emphysema-like lesions occur. The increased numbers of premature senescent cells together with a slower cell proliferation contribute to the thinner Tsept and emphysema-like lesions in the lungs of the progeny of the F0 females that were exposed to nicotine during pregnancy and lactation.

Conclusion: Grand-maternal nicotine exposure induce structural changes in the lungs of the F1 and F2 generations that resembles premature aging.
Thyroid hormone deficiency alters insulin signalling proteins in skeletal muscle of fetal sheep near term

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Introduction: Thyroid hormones are important in the control of normal growth and development before birth. In the ovine fetus, hypothyroidism causes growth retardation and changes in the contractile characteristics of skeletal muscle. This study investigated the effect of thyroidectomy on insulin signalling pathways in skeletal muscle of fetal sheep during late gestation.

Materials and Methods: After maternal and fetal euthanasia (200 mg/kg pentobarbitone iv), umbilical blood and biceps femoralis muscle were collected from sheep fetuses at 130 (n=25) and 144 (n=17) days of gestation. Thirteen fetuses at 130d and ten fetuses at 144d were thyroidectomised previously under halothane anaesthesia at 105-110d. All procedures were carried out under the UK Animals (Scientific Procedures) Act 1986. Plasma thyroxine, tri-iodothyronine, cortisol and insulin were measured by RIA or ELISA. Muscle protein levels of insulin receptor β-subunit (IRβ), insulin-like growth factor type 1 receptor (IGF-1R), phosphorylated protein kinase B (p-Akt-Ser473), Akt1, Akt2, as well as mammalian target of rapamyosin (p-mTOR-Ser2448), p-S6 kinase-Thr389, protein kinase C ζ (PKCζ), calpastatin and GLUT4 were measured by Western blotting. Statistical significance was assessed by two-way ANOVA followed by Tukey test.

Results and Discussion: At both 130 and 144d of gestation, hypothyroidism caused significant increases in muscle protein levels of IGF-1R, Akt1 and p-mTOR. At 144d alone, plasma insulin and muscle p-S6 kinase, PKCζ, calpastatin and GLUT4 were significantly higher, and p-Akt and Akt2 significantly lower, in thyroidectomised compared with intact fetuses. Thyroidectomy had no effect on muscle IRβ at either age.

Conclusion: Thyroid hormones influence the normal expression of metabolic signalling proteins in skeletal muscle of the ovine fetus near term. Some of the changes in skeletal muscle induced by hypothyroidism in utero may be mediated by elevated plasma insulin in late gestation and may have consequences for growth and insulin sensitivity in later life.

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Real-time ultrasound assessment of body and breathing movements and abdominal diameter in fetal lambs from 55 d gestation to term

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Fetal motility in the form of body and breathing movements are important for the development of the musculo-skeletal system and lung, respectively. There have been numerous studies of fetal motility, both in terms of the development of motility in early pregnancy and the subsequent qualitative and quantitative changes with advancing gestation. In terms of the latter changes, real-time ultrasound observations in pregnant humans, pigs, and guinea pigs have indicated a progressive decline of fetal movements and increasing quiescence in late gestation. However, similar data from fetal lambs are limited. Thus the objective was to monitor fetal motility serially in pregnant sheep from 55 days gestation to term. In addition, as all but one of the ewes studied carried twins or triplets, we obtained serial measurements of fetal trunk diameter to determine if fetal growth in these pregnancies differed from the published data of growth of singleton fetuses. We used real-time ultrasound to measure motility and abdominal diameter in fetal lambs at weekly intervals for 30 min from 55 d to term (n=8). Fetal body movement counts/min were relatively constant between 55 and ~90 d, and declined progressively thereafter, a relationship best described by piecewise linear regression with 2 elements. The breakpoint in the regression curves averaged 91.9±5.2 d. The relationship between gestational age and abdominal diameter was also best described by piecewise linear regression. The breakpoint averaged 113.1±3.9 d, following which the rate of abdominal growth declined, and the value was significantly greater than the movement breakpoint. There was a significant linear relationship between the movement and abdominal breakpoints, with the latter occurring 21.6±6.6 d later. These results suggest that both fetal motility and growth may decrease in order to lower fetal \(\text{O}_2\) demands to match the progressive decline in fetal \(\text{O}_2\) delivery with advancing gestation.
Session IV – Brain, part A

Chaired by Dan Rurak & Manon Benders
Perinatal hypoxia impairs synaptic plasticity in the hippocampus of male but not female marmosets later in life

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Introduction: Perinatal hypoxia can result in motor and cognitive impairment later in life. Epidemiological evidence shows that male children are more susceptible to adverse outcomes of hypoxia than females. Current attempts to reduce the impact include reducing the head temperature as soon as possible after the episode of hypoxia. We developed a primate model for exploration of alternative approaches.

Methods: Marmosets were exposed to hypoxia within 24 h of birth. Female/male pairs were placed in a Perspex box (110x70x70cm), within a humidicrib at 34°C, through which flowed gas containing 20% O₂ in N₂ (control) or 3% O₂ in N₂ (hypoxia). After 1 h, the neonates were removed from the boxes and placed within the humidicrib for 30 min before return to the dam. When the infants were 3 months of age (weaning), they were deeply anaesthetized with pentobarbitone, the head rapidly removed into ice-cold artificial cerebrospinal fluid (aCSF) and sagittal brain slices (300μm) cut on a vibratome. Slices of hippocampus were transferred to an organ bath, continuously superfused with warm (35°C) aCSF, and synaptic plasticity was studied electrophysiologically at CA3/CA1 synapses.

Results: Oxygen saturation, recorded continuously via the tail, fell to below 10% in the hypoxia neonates, while it remained at around 98% in controls. Maximal synaptic response, presynaptic facilitation and post synaptic potentiation were significantly greater in control males compared with control females. These variables were entirely resistant to perinatal hypoxia in females. In contrast, maximal synaptic responses, presynaptic facilitation and post synaptic potentiation were all significantly impaired in males that had been exposed to the single episode of hypoxia neonatally.

Conclusions: We have established a non-human primate model that may hold promise for future detailed studies of the effects of perinatal insults on synaptic plasticity in the hippocampus and is suitable for screening new possible therapies.
Pre-existing hypoxia in multiple pregnancies predisposes to systemic compromise, impaired cerebral perfusion, and greater EEG suppression during repeated brief umbilical cord occlusions in near-term fetal sheep

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Introduction: Pre-existing hypoxia particularly in growth restricted fetuses is associated with increased risk of still birth, metabolic acidosis during labour, and abnormal neurodevelopment. However, there are few data on electrophysiological and cerebrovascular adaption to repeated labour-like insults in fetuses with pre-existing hypoxia.

Methods: Chronically instrumented near-term fetal sheep (125 ± 3 days) were subjected to repeated 1-minute umbilical cord occlusions every 5 minutes (normoxia group; PaO$_2$ > 17mmHg, n = 9, pre-existing hypoxia group; PaO$_2$ < 17mmHg, n = 9) for a total of 4 hours or until mean arterial blood pressure fell below 20mm Hg during two successive occlusions. The pre-existing hypoxia group was smaller (3258g ± 599 vs. 4043g ± 373, p<0.01) and contained more twins (7 vs. 4 twins).

Results: Repeated umbilical cord occlusions resulted in progressive metabolic acidosis that was greater in fetuses with pre-existing hypoxia (pH =7.08 ± 0.04 vs. 7.33 ± 0.02, p<0.001) and severe hypotension (24.7 ± 1.6 vs. 51.4 ± 2.8 mmHg, p<0.001) after 4 hours. Five of 9 pre-existing hypoxia fetuses were unable to complete the full series of occlusions (p<0.05). Pre-existing hypoxia was associated with lower intra-occlusion maximum and minimum carotid blood flow (p<0.01), with progressive onset of hypoperfusion (23.6 ± 6.1 vs. 63.0 ± 4.8, p<0.001, last occlusion). Cortical impedance, a measure of cytotoxic edema, was higher (p = 0.05) throughout the occlusions and preliminary analysis suggests greater depression of EEG power.

Conclusion: Pre-existing, stable hypoxia was associated with progressive severe, systemic decompensation during repeated brief labour-like asphyxia, with severe cerebral hypoperfusion, increased cortical cell swelling, and possibly more profound reduction in EEG activity. These results suggest reduced cardiac and cerebral tolerance to labour-like insults.
Hypothalamic global gene expression in response to hypoxia or brachiocephalic occlusion in late-gestation fetal sheep

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This study was designed to identify gene networks in the fetal hypothalamus that are up- or downregulated in response to a 30 minute period of maternal ventilatory hypoxia (decreased fetal PaO2 to ~10 mm Hg) or a 10 min period of cerebral hypoperfusion caused by occlusion of the brachiocephalic artery. Chronically catheterized fetal sheep (130-135 days gestation, term=147d) were euthanized 1 hr after onset of a 30 min hypoxia (n=3) or normoxia (n=3) or 3 hr after onset of a 10 min occlusion (n=4) or sham occlusion (n=4) of the brachiocephalic artery. mRNA was extracted and analyzed using the ovine Agilent 15.5k array, which was annotated in this lab. With respect to hypoxia, 241 genes were significantly (p<0.005) upregulated and 880 genes were significantly downregulated. With respect to BCO, 272 genes were significantly upregulated and 386 downregulated. GO terms associated with upregulated genes after hypoxia included ubiquitination, Cu homeostasis, oxidative metabolism. GO terms associated with downregulated genes after hypoxia included cell cycle, immune development, and apoptosis. KEGG analysis indicated a shift from aerobic to anaerobic metabolism and decreased gene expression in hematopoietic cell lineages. GO terms associated with upregulated genes after BCO include apoptosis, immune responses, post-translational protein modification. GO terms associated with downregulated genes after BCO include humoral immune responses, hormone regulation, estrogen and glucocorticoid signaling. Eighteen genes upregulated by BCO are genes regulated by HIF1α, whereas none of the genes differentially regulated by hypoxia are HIF1α-responsive. We conclude that fetal hypothalamic genomic response to maternal ventilatory hypoxia reflects appropriate responses in cellular energetics but is not likely the result of direct cellular hypoxia. Genomic responses to cerebral hypoperfusion, on the other hand, are likely to be partially the result of activation of HIF1α-responsive genes.
O18

Prenatal hypoxia and neurological disease in adulthood: role of oxidative stress

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Introduction: Research on developmental programming has largely focused on alterations in maternal nutrition and their contributions to cardiometabolic diseases. Few studies have examined developmental programming of neurological disease. Even fewer have investigated hypoxia as the programming stimulus, as occurs during preeclampsia or placental insufficiency [1,2]. We have shown that prenatal hypoxia impairs hippocampal function and structure in adult rat offspring. This programming is linked to oxidative stress, as treatment of hypoxic pregnancies with vitamin C ameliorated the adverse effects. In this interventional study, we have investigated in rats the effects of prenatal hypoxia on anxiety-related behaviour and cognitive function, and determined whether allopurinol conveys neuroprotective effects.

Materials and Methods: Wistar dams were exposed to normoxia (N, 21% O₂) or hypoxia (H, 13% O₂) from days 6-21 of pregnancy +/- allopurinol (HA: 30mg/kg/day in jelly). At 3.5 months, behaviour and cognitive function were assessed using an elevated plus maze and object recognition (OR) task, respectively (n=9-11, one male per litter per group). The OR task assesses the integrity of the perirhinal cortex, which is implicated in Alzheimer’s disease.

Results: At 4 months, body (N: 567±12g; H: 530±13g; HA: 542±22g) and brain (N: 2.05±0.03; H: 2.05±0.04; HA: 2.02±0.03) weights were not different between groups. The measure of OR, the discrimination ratio (DR), was reduced 3 and 24 hours after training in adult offspring of hypoxic pregnancy compared to controls, suggesting memory impairment (Fig.1). These adverse effects were absent in offspring of hypoxic pregnancy treated with allopurinol. Performance in the elevated plus maze was not different between groups.

![Fig. 1. The discrimination ratio (DR) at 3 and 24 hours for the object recognition (OR) task. Values are mean ± SEM in normoxic (■). hypoxic (■) and hypoxic + allo (■) offspring. Significant differences * P<0.05, one-way ANOVA with post hoc Tukey.](image-url)
O18

**Conclusion:** Memory impairment in adulthood can be programmed by prenatal hypoxia. Treatment with antioxidants of hypoxic pregnancies improves cognitive function and cerebral structure in the adult offspring. Oxidative stress provides a link between prenatal hypoxia and the developmental programming of neurodegenerative disease.

**References**
Connexin 43 hemichannel blockade: mimetic peptide dose response after ischaemia

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Introduction: We have previously shown that blockade of connexin 43 hemichannels with a specific mimetic peptide reduced the occurrence of seizures, improved recovery of EEG power and reduced loss of oligodendrocytes following global cerebral ischaemia in the near-term fetal sheep. In the current study we aimed to evaluate the dose response.

Materials & Methods: Near-term fetal sheep (0.85 gestation) received 30 min carotid artery occlusion. Two doses of mimetic peptide (50 µmol/kg/h (high dose, n=6) or 50 µmol/kg/h for one hour followed by 50 µmol/kg/24h (low dose, n=6)) or vehicle was infused I.C.V. for 25 hours starting from 90 min after ischaemia or vehicle only in the control group (n = 7).

Results and discussion: Peptide infusion at both doses was associated with a significant increase in EEG power, 2-12 hours after ischaemia, compared to control (p<0.05). In the ischaemia-high dose group, impedance was significantly increased between 18-32 hours (p<0.05). Arterial lactate was lowest in the ischaemia-low dose group (2.33 ± 0.45) compared to controls (4.7 ± 1.09 mmol/L); a trend towards increased lactate was seen in the ischaemia-high-dose group (10.21 ± 3.26 mmol/L) one day after ischaemia. Compared to control and low dose group, there was a trend towards increased fetal death in the ischaemia-high dose group (0/7, 0/6 and 2/6 respectively). A significant increase in EEG power was seen from day five in the ischaemia-low dose group (p<0.05). Whilst blocking hemichannels with the low dose infusion appeared to reduce injury, high dose mimetic peptide may also block gap junctions, causing dysfunction of the astrocytic syncitium and increased injury.

Conclusions: This study demonstrated that low dose mimetic peptide infusion resulted in a better outcome, whilst high dose mimetic peptide infusion exacerbated injury, following ischaemia.
Insulin-like growth factor (IGF)-1 increases gliosis but reduces caspase-3 activation in grey matter after ischemia in near-term fetal sheep

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Introduction: White matter protection with exogenous infusion of insulin like growth factor (IGF-1) is associated with marked gliosis in the fetal sheep. Pathologically, gliosis is associated with adverse outcome. We therefore investigated the dose response for grey matter protection after ischemia in near-term fetal sheep, and how this was related to the glial response.

Material and Methods: Near-term fetal sheep received reversible cerebral ischemia induced by complete occlusion of both carotid arteries for 30 minutes (n=25), or sham occlusion (n=6). 90 minutes after ischemia, fetuses were randomized to receive either 3μg (n=10) or 30μg IGF-1 (n=8) or vehicle (1ml artificial CSF) (n=7) infused over 1 h. Fetuses were killed after 4 days recovery. Neuronal damage was assessed in the cortex, hippocampus, striatum and thalamus. The relationship between gliosis and cell survival was assessed in the temporal lobe, a key penumbral region, using NeuN immunoreactivity to identify surviving neurons, IB4 for reactive microglia, glial fibrillary acidic protein (GFAP) for astrocytes, proliferating cell nuclear antigen (PCNA) for proliferating cells and Caspase-3 ASP-175 as a marker of ongoing apoptosis.

Results: Cerebral ischemia was associated with severe neuronal loss in the cortex, hippocampal regions, striatum and thalamus (P<0.0001). A single infusion of 3μg of IGF-1 but not 30μg was associated with a significant reduction in neuronal damage (p<0.05), and numbers of Caspase-3 positive neurons (p<0.05). In the temporal cortex, ischemia was associated with a significant increase in proliferating cells (P<0.05), including astrocytes (p<0.05) and microglia (p<0.05). 3 μg IGF-1 was associated with a further increase in astrocytes but a relative reduction in numbers of microglia and caspase-3 positive cells.

Conclusions: In near-term fetal sheep neuroprotection with low-dose IGF-1 was associated with greater astrogliosis but attenuation of the inflammatory reaction and apoptosis. Speculatively, reactive glia may contribute to IGF-1 mediated grey matter protection.
O21

Exposure to maternal glucocorticoids exacerbates post-asphyxial brain injury in the preterm fetus

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Introduction: Maternal glucocorticoid treatment for threatened premature delivery consistently improves survival and short-term morbidity; however, its effects on neurodevelopmental outcome are rather variable. In sheep, maternal dexamethasone leads to dramatic, evolving hyperactivity in the preterm fetus,1 raising the possibility that dexamethasone may adversely affect neural suppression which is an important protective response to hypoxic-ischaemic injury. We tested the hypothesis that maternal glucocorticoid exposure could sensitise the preterm brain to asphyxia-induced injury.

Methods: Chronically instrumented fetal sheep at 0.7 of gestation received asphyxia induced by complete umbilical cord occlusion for 25 minutes. 15 minutes after release of occlusion, ewes received an i.m. injection of either dexamethasone (12 mg in 3 ml saline, n=6) or saline (n = 7). Sheep were killed 7 days later for histology.

Results: Maternal dexamethasone was associated with transient hyperglycaemia (peak 3.5±0.2 vs 1.4±0.2mmol/L at 6 h), reduced suppression of the EEG activity in the first 24 h after occlusion (maximum recovery -1.5 ± 1.2 dB vs -5.0 ± 1.4 dB in controls, p<0.01), with increased epileptiform transient activity on the continuous EEG recordings (peak: 31 ± 8 % of activity vs. 13 ± 5 %). This was associated with significant increase in neuronal loss in the hippocampus (CA1/2: 51 vs. 1.1±1.1, CA3 35±12 VS 13±6.3).

Conclusions: These data strongly suggest that maternal dexamethasone therapy has potential to moderately exacerbate brain damage in an already compromised fetus. The precise mechanisms are unknown but may include fetal hyperglycaemia and loss of post-asphyxial metabolic suppression.

Session V – Miscellaneous

Chaired by Keiji Suzuki & Luc Zimmermann
Ovine fetal thymus response to lipopolysaccharide-induced chorioamnionitis and antenatal corticosteroids

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Introduction: Chorioamnionitis is associated with preterm delivery and involution of the fetal thymus. Women at risk of preterm delivery receive antenatal corticosteroids which improve neonatal outcome. However, the effects of antenatal corticosteroids on the fetal thymus in the settings of chorioamnionitis are unknown. Sonic hedgehog (Shh) and Bone morphogenetic protein (BMP) are inhibitors of T-cell development and are sensitive to prenatal events. We hypothesized that intra-uterine exposure to lipopolysaccharide (LPS) and antenatal corticosteroids would alter fetal thymic structure and T-cell development by modulating Shh and BMP4.

Materials and Methods: Time-mated ewes received an intra-amniotic injection of LPS and/or maternal intra-muscular betamethasone, or saline as a control, 7 and/or 14 days (n=5-7) before delivery at 120 days gestational age (term=150d). The fetal intra-thoracic thymus was evaluated for indicators of thymic development.

Results: Intra-amniotic LPS decreased the cortico-medullary ratio of the thymus, but increased Toll-like receptor (TLR) 4 mRNA significantly by 2-fold and CD3 expression by 3-fold 7 days after exposure, indicating involution and activation of the fetal thymus. This activation response was accompanied by a 60% decrease in Shh and BMP4 mRNA, suggesting increased differentiation of T-cells. TLR4 mRNA and CD3 expression remained significantly elevated 14 days after the LPS exposure, although the number of Foxp3-positive cells decreased by 40%. Betamethasone treatment 7 days after the LPS exposure did not prevent the LPS-induced changes in thymic structure and activation. Betamethasone treatment 7 days before LPS exposure prevented thymic activation but not structural changes.

Conclusion: Intra-uterine LPS exposure activated the fetal thymus with changes in structure and a persistent decrease in Foxp3-positive T-cells which may alter the function of the immune system. Only antenatal corticosteroid administration before the inflammatory stimulus prevented these effects on the thymus. This study illustrates the complicated interactions of pro- and anti-inflammatory stimuli on fetal immune development.
Effects of prenatal LPS exposure on postnatal lung function in rats

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Introduction: Chorioamnionitis is one of the major causes of preterm delivery. It is also known to be associated with development of chronic lung disease. The aim was to study how fetal exposure of lipopolysaccharide (LPS) affects on postnatal lung function in rats. Our hypothesis was that fetal LPS exposure would cause persistent alterations in lung function and airway responsiveness.

Materials and Methods: At 20 d gestation, pregnant SD rats were anesthetized and the uterus exposed under general anesthesia. The uterine wall was punctured and 0.1mL saline (SAL group) or 1 μg LPS; E. coli endotoxin (O55:B5; Sigma, St. Louis, MO, USA) dissolved in 0.1mL saline (LPS group) injected into each amniotic sac. Pups without operation served as controls (NTX group). At 22 d (term), the fetuses were delivered vaginally or abdominally. The newborn pups were nursed by their own or foster mother. At 3 weeks, the pups were anesthetized and ventilated for lung function measurement (resistance and compliance of the respiratory system; Rrs and Crs, respectively) using flexiVent system® (SCIREQ, Montreal, QC, Canada). Response to inhaled methacholine was also examined.

Results and Discussion: LPS-exposed pups had the highest perinatal mortality rate among the 3 groups (LPS 75%, SAL 15%, NTX 1%; p<0.01). Baseline Rrs and Crs were not different between groups. However, in response to methacholine, both increase in Rrs and decrease in Crs were significantly enhanced in the LPS group. Altered responsiveness to inhaled methacholine indicates impaired lung function representing mild form of chronic lung disease.

Conclusion: Prenatal exposure of amniotic LPS resulted in higher perinatal mortality but relatively modest influence on postnatal growth in the surviving offspring. However, it also affected long term lung function in terms of responsiveness to cholinergic stimulation.
Hypoxia development in patients with transposition of the great arteries according to timing of arterial switch operation

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Introduction: transposition of the great arteries (TGA) is compatible with fetal survival and normal development because of hemodynamic features. After birth, starting of lung oxygenation and closure of the fetal shunts, TGA manifest by development of critical hypoxia. In the case of prenatal diagnoses of TGA, we propose to perform arterial switch operation (ASO) at the first hours of patient’s life while fetal shunts are still presence. This study was designed to test the hypothesis that ASO at the first hours of patient’s life allows preventing development of deep hypoxia.

Materials and Methods: from December 2009 to March 2012, 174 consecutive neonates underwent ASO at our institution. Twenty one of them with different timing of surgery were allocated into two groups for investigation of hypoxia development: study group (n=10) with prenatally diagnoses and ASO at the first hours of life; control group (n=11) with postnatal diagnoses, balloon atrioseptostomy and delayed ASO (conventional approach). Levels of hypoxia-inducible factor-1α (HIF-1α) were measured in peripheral blood just before ASO, at the 1st, 3rd and 7th days postoperatively. During the surgical procedure, before CPB a biopsy from the right atrium was taken for light (LM) and electron microscopy (EM).

Results and Discussion: The groups were similar in diagnoses, birth weight, cardiopulmonary bypass protocol and surgery technique. The mean age at operation in the study group was 3.8±1.1 hrs vs 183±46 hrs in control. Preoperative levels of HIF-1α were significantly elevated in patients of control group compared with a study group and decreased postoperatively. Muscular contractures, sarcoplastic vacuolation during LM, and abundance of ANF during EM were typical for myocardium of newborns of control as opposed to study group.

Conclusion: Delayed ASO is associated with elevated levels of HIF-1α and signs of hypoxic injury of myocardium in neonates with TGA before surgery.
O25

Placental origins of later cancer risk

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Introduction: There is increasing evidence that chronic degenerative diseases originate in utero. Small size at birth is associated with increased rates of cardiovascular disease, type 2 diabetes, hypertension, osteoporosis and depression in later life. Higher birth weights are associated with increased cancer risk. Recently, associations between a large size of the placental surface and lung cancer have been found in a study in Finland. Here, we examine the association between placental size and later cancer risk.

Materials and Methods: The Dutch Famine Birth Cohort consists of 2414 term singletons born alive in the Wilhelmina Gasthuis in Amsterdam between 1 November 1943 and 28 February 1947. Medical birth records have been preserved, providing information about the mother, the size of the baby and placenta at birth. The cohort has been traced and 2254 members could be linked to the mortality registry. Causes of death until December 31st 2007 were provided by linking the cohort with Statistics Netherlands. In total, 98 cohort members died of cancer. Using Cox proportional hazard regression, we examined associations between placental size at birth and cancer mortality.

Results and Discussion: Placental size was associated with cancer mortality, but birth weight was not. Hazard ratios (HR) for cancer rose, as the surface area of the placenta at birth increased. For every 40 cm² increase in placental area the HR for cancer was 1.19 (1.06 to 1.33; P = 0.003), when adjusted for sex. This association was similar for men and women, and was not affected by prenatal famine exposure.

Conclusion: Our study shows that a large placental surface area is associated with increased cancer mortality. This adds to the evidence that cancer might originate in utero. The underlying biological mechanism for this phenomenon remains to be elucidated.
O26

Treatment of placental pathological hypoxia by administration of oxygen-loaded nanobubbles: Preliminary studies

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Introduction: Placental hypoxia is responsible for impaired fetal growth. Although it can be diagnosed during early pregnancy, there are no therapeutic strategies to avoid hypoxia-related damages except for timed premature delivery. Herein, we tested new specific performances of the oxygen-loaded chitosan and dextran nanobubbles (OLNs) on human chorionic villous explants in order to optimize selective oxygen delivery to treat placental-hypoxia. Moreover, using in-vitro models, we investigated the feasibility of OLNs transdermal administration by sonication.

Materials and methods: OLNs were prepared as previously described (1). Human villous explants were obtained from term placentae. 500 µl of OLNs were delivered to the explants cultures after 8h conditioning in normal culture (20% pO2) or hypoxic (3% pO2) conditions. Hypoxia Inducible Factor-1α (HIF-1α), Vascular Endothelial Growth Factor (VEGF) and Macrophage-migration Inhibitory Factor (MIF) mRNA and protein levels were assessed by Real Time PCR and Western Blot. OLNs Sonoporation was induced by 2.5 MHz transducer delivering 5W acoustic power across polyacrylamide and natural (pig skin) membranes and evaluated by measuring O2 concentration across the membranes.

Results: OLNs actively modulated the expression of HIF-1α. In normal culture conditions, the additional O2 supplementation by OLNs administration was accompanied by evidences of oxidative stress. VEGF and MIF mRNA and protein levels showed a dose-dependent reduction following post-hypoxic OLNs administration. The sonophoretic transport was achieved. Its efficiency was related to the duration of sonication (with a consequent increase in temperature) and to the initial difference of oxygen concentration across the membrane.

Conclusions: Our data show that OLNs could actively revert hypoxia in human villous explants by modulating the expression of the main factors involved in placental hypoxia-related damage typical of fetal growth impairment. Looking forward for non-invasive therapeutic approaches, transmembrane sonoporation seems a promising tool, although the related temperature increase must be kept under control.

Effect of SSRI exposure on fetal general movements, a case-control study

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⁴ Department of Clinical Pharmacy, University Medical Center Utrecht, the Netherlands

Introduction: Selective serotonin reuptake inhibitors (SSRIs) are often prescribed during pregnancy. Preliminary studies suggest influence on the quality and quantity of fetal general movements (1,2). The aim of our study is to investigate whether the use of SSRIs affects the quality of fetal general movements during the three trimesters of pregnancy. We hypothesize that SSRI exposure affects the quality of the fetal GMs.

Materials and Methods: Longitudinal case-control study. This study is part of an on-going study and has been approved by the Utrecht Ethics Committee(2). Sixty women were included at University Medical Centre Utrecht. The participants had used SSRIs for >6 months prior to their pregnancy. Ten minutes assessments on fetal general movements were analysed randomly from 60 min. ultrasound observations made at 15-19 wk (T1), 27-29 wk (T2) and 120 min at 37-39 wk (T3). Behavioural states 1F and 2F were distinguished at T3 by means of fetal motility, eye movements, and heart rate. The quality of general movements was examined for variation in speed, amplitude, complexity (direction and participation of body parts), fluency, waxing and waning activity, resulting in an overall impression of normal or abnormal GMs.

Results: The study group comprised 13 controls; 13 women who discontinued medication before conception or during the first trimester (stoppers); 20 women using standard dosage; and 12 using high dosage SSRIs.

Compared with controls, SSRI-exposed foetuses showed similar quality of GMs at T1 and T2 and during T3 behavioural state 2F (active sleep). However, foetuses exposed to SSRIs throughout pregnancy and even those of stoppers exhibited increased complexity and waxing and waning during T3 behavioural state 1F (quiet sleep) as compared to controls (both p<0.05).

Conclusion: SSRI exposure affects the quality aspects of general movements near term during the quiet behavioural state. The effect is not dosage related and also seen half a year after SSRI cessation.

Session VI – Stress

Chaired by Bea Van den Berg & Eduard Mulder
O28

Prenatal maternal psychosomatic stress: effects on fetal brain development following maternal neurosteroid treatment in guinea pigs

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² School of Biomedical Science, Newcastle University, Australia. ³ Richie Centre for Baby Health, Monash University, Clayton, Australia

Introduction: Prenatal maternal psychosomatic stress has been associated with many detrimental perinatal outcomes, including adverse effect on fetal brain development. We have previously shown repeated glucocorticoid administration perturbs neurosteroid synthesis in the fetal brain, causing decreased myelination. These findings suggest a critical role for neurosteroids in mediating the effects of prenatal stress on the fetal brain. Our aim was to first determine the effect of prenatal stress on the late gestational fetal brain and then to assess the effectiveness of maternal allopregnanolone administration in ameliorating these effects on the brain.

Methods: Stress was induced by exposing pregnant guinea pigs to strobe light for 2h/day on gestational day 50, 55, 60, 65 (term 70d). Maternal salivary cortisol was measured before and after each stress event. Exogenous neurosteroid therapy was administered to dams subcutaneously on gestational day 60-68. Fetal brains were collected at term for immunohistochemical analysis of markers of myelination (MBP), reactive astrocytes (GFAP) and mature neurons (MAP2). Allopregnanolone levels were measured in fetal plasma at term.

Results: Salivary cortisol concentrations were significantly increased with each stress event. Female fetuses demonstrated higher brain to liver ratios, indicative of brain sparing. Male fetuses showed significantly reduced MBP and GFAP in the hippocampus (CA1 p<0.001) and cortex (p<0.05) indicating compromised brain growth. Both male and female fetuses showed reduced MAP2 in the hippocampus (CA1 p<0.001). Maternal allopregnanolone administration raised levels in fetal plasma in control pregnancies, but not in stressed pregnancies.

Conclusions: These results show that prenatal stress has detrimental effects on brain development in male fetuses; whereas female fetuses are less susceptible to the effects of stress. In addition, while allopregnanolone treatment of dams raised fetal levels in controls, this increase was not seen in prenatal stressed fetuses, suggesting a novel mechanism of dysregulation of neurosteroidogenesis in prenatally stressed pregnancies.
O29

Chronic maternal stress during pregnancy potentiates stress-mediated decrease of uterine blood flow

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Introduction: Acute stress in pregnant sheep leads to a catecholamine mediated decrease in uterine blood flow (UBF) and induces prolonged fetal lactate increase and decrease in oxygen saturation. In agreement with, chronic exposure to maternal stress induces fetal growth retardation in sheep (Frauendorf, Reprod Sci, 2011) and humans (Rondo, Eur J Clin Nutr, 2003). We hypothesized, chronic maternal stress attenuates the UBF decrease and fetal lactacidosis due to adaptation to the stressor.

Methods: Ten pregnant sheep underwent repeated isolation stress between 0.2 and 0.66 gestation (30 and 100 days gestation age, dGA, term 150 days) resulting in a reproducible cortisol increase with only slight habituation. Ten pregnant ewes functioned as controls. Five stressed and five control animals were chronically instrumented with maternal and fetal catheters into the carotid artery and the jugular vein and an uterine ultrasound flow probe five days before acute isolation stress at 0.75 or 0.87 gestation (110 or 130 dGA).

Results: Acute maternal stress at 0.75 and 0.87 gestation transiently increased maternal blood pressure (MBP) and maternal heart rate (MHR) and decreased UBF (p<0.05, Table 1). UBF decrease was prolonged in chronically stressed ewes at both gestational ages (p<0.05, Table 1). Fetuses in all groups responded with an increase in lactate (p<0.05). There was no drop in fetal pH and oxygen saturation at 0.75 gestation. At 0.87 gestation, fetal pH and oxygen saturation decreased. Preceding stress prevented the drop in pH (p<0.05) probably because of hyperventilation-mediated maternal hypocapnia.

Conclusions: Maternal stress induces a decrease in UBF during the third trimester that is more prolonged with gestational age. The UBF decrease is potentiated by preceding chronic stress during the first and second trimester and persists for 4 weeks after discontinuation of chronic stress.

<table>
<thead>
<tr>
<th></th>
<th>MBP increase (mmHg)</th>
<th>MHR increase (bpm)</th>
<th>UBF decrease (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls 0.75 gestation</td>
<td>81.9±1.5 to 96.2±2.5 for 8 min*</td>
<td>88.3±2.34 to 110.3±5.8 for 30 min*</td>
<td>13.7±4.0% for 12 min*</td>
</tr>
<tr>
<td>Stress 0.75 gestation</td>
<td>87.3±7.5 to 97.5±5.2 for 22 min*</td>
<td>87.8±11.2 to 109.0±11.5 for 4 min (n=3)</td>
<td>19.5±6.7% for 85 min*$</td>
</tr>
<tr>
<td>Controls 0.87 gestation</td>
<td>83±2.7 to 89±3.9 for 9 min*</td>
<td>98±4.7 to 125.9±6.2 for 30 min*</td>
<td>11.8±2.7% for 75 min*</td>
</tr>
<tr>
<td>Stress 0.87 gestation</td>
<td>89.1±2.0 to 97.8±3.8 for 14 min*</td>
<td>103.2±6.3 to 115.9±9.4 for 65 min*</td>
<td>11.8±3.5% for 120 min*$</td>
</tr>
</tbody>
</table>

Table 1: Changes in MBP, MHR and UBF at 0.75 and 0.87 gestation in controls and stressed ewes (mean±SEM; n=5; *p<0.05 compared to baseline, $p<0.05 compared to controls).
Does maternal psychological distress in second trimester of pregnancy affect feto-placental volume blood flow in third trimester?

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**Introduction:** Maternal psychological distress in pregnancy has been linked to lower birthweight. Type and timing of distress seems to play a role. Reduced placental blood flow has been suggested as a mechanism. We examined the association of psychological distress in the first half of pregnancy and feto-placental volume blood flow in third trimester.

**Materials and Methods:** The study group consisted of 61 pregnant women with a structural malformation in a previous child or fetus, and a comparison group of 94 women without history of congenital anomaly. All participants had normal findings on ultrasound examination. Psychological distress was assessed in second trimester (median 18 weeks, range 12–24), using psychometric questionnaires: the General Health Questionnaire-28 (Depression and Anxiety subscales), Edinburgh Postnatal Depression Scale (EPDS), and Impact of Event Scale-22 (relating to “the condition of the fetus”; subscales Intrusion, Avoidance and Arousal). At 30 (SD 0.6) weeks of gestation, blood flow velocity and vessel diameter in the intra-abdominal portion of the umbilical vein (UV) were obtained using ultrasound. Volume flow was calculated and normalized for fetal abdominal circumference (UVAC, ml/min/cm).

**Results and Discussion:** Distress scores were significantly higher in the study group (P<0.001). Distress was not related to gestational age. In the study group, all distress measures but Avoidance correlated positively with UVAC at 30 weeks. In the comparison group, EPDS and Anxiety correlated negatively with UVAC at borderline significance (see Table 1). Maternal age, parity, body mass index at 30 weeks, and smoking were not related to UVAC, and showed no significant effect in multiple regression analyses.

**Conclusion:** In pregnant women with a history of congenital malformation, several types of psychological distress measures in second trimester were associated with increased normalized feto-placental blood flow at 30 weeks. In a group without previous malformation, a possible opposite effect was found.
Table 1. Correlations between distress measures and Ln-transformed umbilical vein volume blood flow, normalized for fetal abdominal circumference (Ln-UVAC, ml/min/cm).

<table>
<thead>
<tr>
<th>Distress measure</th>
<th>previous anomaly (n = 61)</th>
<th>no previous anomaly (n = 94)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spearman’s rho</td>
<td>P</td>
</tr>
<tr>
<td>GHQ Depression</td>
<td>0.26</td>
<td>0.042</td>
</tr>
<tr>
<td>GHQ Anxiety</td>
<td>0.27</td>
<td>0.034</td>
</tr>
<tr>
<td>EPDS</td>
<td>0.36</td>
<td>0.004</td>
</tr>
<tr>
<td>IES Intrusion</td>
<td>0.34</td>
<td>0.007</td>
</tr>
<tr>
<td>IES Avoidance</td>
<td>0.12</td>
<td>0.36</td>
</tr>
<tr>
<td>IES Arousal</td>
<td>0.33</td>
<td>0.010</td>
</tr>
</tbody>
</table>

GHQ, General Health Questionnaire; EPDS, Edinburgh Postnatal Depression Scale; IES, Impact of Event Scale.
O31

Parents’ depressive symptoms during pregnancy and postpartum: effects on infant neonatal neurobehavioural development and temperament in twins

Iva Tendais, Bárbara Figueiredo, and Catarina Canário

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**Introduction:** There is some evidence that parents of twins may be at increased risk for depression, especially mothers at the postpartum. However, little is known about the effects of parents’ depression on twins’ neurobehavioral development and temperament. The objective of this study was to examine the relationship between parents’ depressive symptoms during pregnancy and postpartum and twins’ neonatal neurobehavioral development and temperament and the moderating role of infant gender.

**Materials and Methods:** 45 couples expecting twins completed the Edinburgh Postnatal Depression Scale at each pregnancy trimester, and after birth at 37, 40 and 44 weeks from conception. The Neonatal Behavioral Assessment Scale was administered at each of the neonatal time points. At three months of corrected age, mothers also filled in the Infant Behavior Questionnaire-Revised. The Actor-Partner Interdependence Model (APIM) was used to determine how twins’ outcomes were influenced by each parent.

**Results and Discussion:** Infant gender moderated the relationship between mothers’ and fathers’ depressive symptoms and neonatal neurobehavioral development and temperament. Depressive symptoms at early pregnancy predicted lower habituation on female twins. The increase of depressive symptoms during pregnancy predicted lower scores on habituation for female twins but higher scores for male twins. Depressive symptoms at early postpartum predicted more negative affectivity and less orienting/regulation on female twins.

**Conclusion:** Mothers’ and fathers’ depressive symptoms seem to have an impact on neurobehavioral development and temperament of twins mainly on female twins. This study contributes to the body of evidence on the maternal and paternal effects of depression on child outcomes.
Parents’ depressive symptoms during pregnancy and postpartum: effects on toddler’s behavior problems

Catarina Canário and Bárbara Figueiredo

University of Minho, Braga, Portugal

Introduction: Mothers’ and fathers’ depressive symptoms have been identified in previous studies as risk factors for internalizing, externalizing and total behavior problems in children. However, little is known about the effects of parents’ depressive symptoms during pregnancy and postpartum on toddler’s behavior problems. This study aimed to assess the impact of mother’s and father’s depressive symptoms during pregnancy and postpartum on toddler’s behavior problems (internalizing, externalizing and total behavior problems).

Materials and Methods: 129 couples (N=258), recruited in a maternity hospital, completed the Edinburgh Postnatal Depression Scale (EPDS) at each pregnancy trimester, childbirth, three and 30 months postpartum. At this last time point, each parent also filled in the Children Behavior Checklist (CBCL 1½ - 5 years old). The Actor-Partner Interdependence Model (APIM) was used to determine how outcomes were influenced by both members of the dyad (mother and father).

Results and Discussion: The increase of mothers’ and fathers’ depressive symptoms during pregnancy accounted for toddlers’ internalizing, externalizing and total behavior problems, whereas the increase of fathers’ depressive symptoms during postpartum accounted for toddlers’ externalizing and total behavior problems. Fathers whose wives had increased depressive symptoms at the first pregnancy trimester identified more externalizing and total behavior problems in toddlers. Mothers and fathers whose spouses had increased depressive symptoms at the first pregnancy trimester identified more internalizing problems in girls.

Conclusion: Mothers’ depressive symptoms during pregnancy and fathers’ depressive symptoms during pregnancy and over postpartum were found to predict toddlers’ behavior problems. As well, depressive symptoms in both dyad members at the first pregnancy trimester demonstrated to be risk factors for toddlers’ behavior problems. Interventions to reduce depressive symptoms in parents since early pregnancy and throughout postpartum should be developed to prevent toddlers’ behavior problems.
Cortisol independent transfer of maternal stress effects to the fetus

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Introduction: Maternal stress during pregnancy induces fetal growth retardation and programs neuropsychiatric diseases in later life (Van den Bergh, Neurosci Biobehav Rev, 2005 / Beydoun, Paediatr Perinat Epidemiol, 2008). It is assumed that these effects are mediated by maternal cortisol which crosses the placenta and programs hyperactivity of the fetal hypothalamo pituitary adrenal axis (HPAA). However, early stress has the most pronounced effects (Rakers, Stress, 2012) when glucocorticoid receptors are not expressed yet (Yang, Endocrinology, 1990) and the fetal HPAA is still inactive. We hypothesized that maternal catecholamines, although they virtually do not cross the placenta directly, have major effects on the fetus by decreasing uterine blood flow (UBF).

Materials and Methods: Five pregnant ewes were chronically instrumented at 125 dGA (days gestational age, term 150 dGA) with maternal and fetal catheters inserted into the carotid artery and the jugular vein and an uterine ultrasound flow probe. At 130 dGA, animals were stressed by isolation for 2h before and after an infusion of labetalol, a mixed alpha and beta adrenergic antagonist.

Results and Discussion: Ewes responded to the isolation stress with an increase in maternal blood pressure (MBP) from 83±2.7 to 89±3.9 mmHg (mean ± SEM) for 9 min (p<0.05), an increase in maternal heart rate (MHR) from 98±4.7 to 126.9±6.2 beats per minute (bpm) for 30 min (p<0.05) and a transient hyperventilation mediated hypocapnia (p<0.05) for 15 min. UBF decreased by 11.8±2.7 % for 75 min (p<0.05) with a maximum of 19±3 % reflecting uterine vasoconstriction. The fetus responded with a delayed and prolonged decrease of pH from 7.39±0.01 to 7.36±0.01, increase of lactate from 1.5±0.2 to 1.8±0.2 mmol/L and decrease of oxygen saturation from 75±2.5 to 67±3.6 % starting at 60 min of isolation (p<0.05). Labetalol infusion led to a decrease of MBP from 72±1.8 to 64±2.9 mmHg, MHR from 104±8.9 to 91±6.2 bpm and UBF by 23±7.6 % (p<0.05). Isolation stress increased MBP and MHR in tendency and UBF by 11±3.4 % (p<0.05) over the entire period of isolation. The latter is a consequence of increased cardiac output in the presence of absent vasoconstriction.

Conclusion: Maternal stress in pregnant sheep induces a catecholamine mediated UBF decrease which is followed by a prolonged fetal lactate increase and decrease in oxygen saturation that may contribute to fetal growth retardation and programming of diseases in later life.
Session VII – Preterm Birth, Lungs & Placenta

Chaired by Alison Forhead & Graham Jenkin
The guinea pig model of human parturition

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² Mothers and Babies Research Centre, University of Newcastle,
³ Hunter Medical Research Institute, Newcastle, New South Wales Australia

The mechanism determining gestational length in women is poorly understood. A major problem is that parturition occurs in the best-studied experimental animals such as mice and rats by a different process than in humans. The guinea pig appears an exception, exhibiting fundamental similarities to women including luteo-placental shift in progesterone production, parturition without falling maternal plasma progesterone and increasing estrogen levels and inefficiency of progesterone to extend gestation beyond term. Maintenance of pregnancy requires progesterone, and prostaglandins induce abortion at any gestational age, like in women. We explored the regulation of parturition in guinea pigs by progesterone and prostaglandins at the molecular level, since the results may establish this animal as a model organism to test interventions to prevent premature birth.

We determined the expression of the key prostaglandin biosynthetic and metabolic enzymes in guinea pig fetal membranes, placenta and myometrium during gestation using quantitative RT-PCR, immunoblotting and immunohistochemistry. We measured progesterone receptor-A, -B and estrogen receptor levels in the myometrium with advancing gestation and determined the effect of exogenous prostaglandin and prostaglandin synthesis inhibitor on myometrial steroid receptor levels.

The prostaglandin-producing capacity of the fetal membranes increased sharply at term by prostaglandin synthase-1 induction in the amnion and prostaglandin dehydrogenase repression in the adjacent fetal membrane. Inhibiting prostaglandin synthase-1 delayed birth. Both progesterone receptor isoforms were repressed in the myometrium at term; estrogen receptor-alpha levels remained unchanged. Exogenous prostaglandin accelerated, while prostaglandin synthase-1 inhibitor delayed the loss of myometrial progesterone receptors shortening and lengthening gestation, respectively.

Thus, increasing prostaglandin output from the fetal membranes is critical for triggering birth. Functional progesterone withdrawal occurs at labour by decreasing myometrial progesterone receptor expression caused, partially at least, by prostaglandins. The guinea pig is an excellent non-primate model of human parturition exhibiting close analogy of the hormonal regulation of birth with humans.
Human labour is associated with decreased myometrial ether-a-go-go related gene (hERG) potassium channels that modulate contractility

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Mothers & Babies Research Centre, John Hunter Hospital, New Lambton, NSW 2305, Australia
Dept Perinatal Medicine, Pregnancy Research Centre, Royal Women’s Hospital, Parkville, Vic 3052. Australia.

Introduction: In human myometrium, contraction is underpinned by a complex action potential (AP) consisting of spikes followed by a plateau of depolarization that controls the duration of contraction. This AP has similarities to the AP in heart. hERG channels play a pivotal role in cardiac contraction by determining the duration of the AP. We hypothesised that hERG channels contribute to the myometrial AP and probed the possibility of its involvement in the change in myometrial contractility during labour.

Methods: Myometrial samples were obtained from consenting women at term, prior to or after the onset of labour and prepared for protein analysis, contractility and electrophysiology studies.

Results: In myometrial strips from non-labouring women hERG expression was readily detectable and hERG inhibitors dofetilide and E-4031 caused a marked increase in the duration of the AP plateau, which was associated with an increase in contraction duration. hERG inhibition also produced additional hyperpolarization between APs, which increased the recovery period between APs and decreased contraction frequency. Currents typical of hERG occurred in isolated patch-clamped myometrial cells. Tissues from women with an increased BMI, who are known to labour poorly, had heightened responses to hERG inhibition. During established labour, hERG expression, the effectiveness of blockers on contractility, and hERG current were markedly reduced.

Conclusions: hERG channel expression occurs in myometrium in pregnant women, and this channel promotes brief APs and contraction. The marked fall in hERG expression and current during labour, strongly suggests that the fall in hERG contributes to the mechanisms that produce the powerful, sustained and well-spaced contractions typical of labour. hERG blockade increases the duration of contraction. Women with high BMI have an exaggerated contractile response to hERG blockers suggesting enhanced hERG levels/activity and this likely contributes to the poor labour in many obese women necessitating caesarean delivery.
O36

Placental mitochondrial uncoupling protein 2 and perinatal inflammation: exposing the preterm infant to increased reactive oxygen species?

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Introduction: Under normal conditions, a delicate balance exists between the production of reactive oxygen species (ROS) and the anti-oxidant defences that protect the human placenta and fetus. The mitochondrial respiratory chain is the major source of ROS in most mammalian cells. Mitochondrial uncoupling protein 2 (UCP2) plays a critical role in the control of mitochondrial ROS production, and has also been implicated in regulating immune activity. Given that we have recently reported sex-specific alterations in placental ROS production in preterm neonates in response to antenatal betamethasone exposure, we aimed to examine placental UCP2 expression to elucidate mitochondrial processes that potentially contribute to poor perinatal outcomes in preterm infants.

Materials and Methods: Relative UCP2 mRNA expression was assessed in the placenta of very preterm (24-28 weeks; n=23), preterm (29-36 weeks; n=19) and term (37-41 weeks; n=11) neonates. Placental ROS production was assessed by measures of lipid peroxidation and nitrative stress in placental tissue homogenates. Arterial and venous cord blood TNFα levels were determined using ELISA. Histological chorioamnionitis was recorded from placental pathology reports following all preterm deliveries. Antenatal steroid administration, birth weight, infant sex and mode of delivery were recorded.

Results and discussion: Placental UCP2 expression increased significantly with gestation (p=0.015) and was unaffected by infant sex or steroid exposure. Placental UCP2 expression was significantly reduced in small for gestational age (SGA) infants, irrespective of prematurity (p<0.05) and was decreased in pregnancies with choriomnionitis (p=0.006). UCP2 expression inversely correlated with arterial cord blood TNFα levels (r=-0.41), but had no relationship with measures of lipid peroxidation or nitrative stress.

Conclusion: Exposure of the developing fetus to increased placental ROS is thought to contribute to the development of morbidities commonly associated with preterm birth. Our current data adds mechanistic support to this theory, with reduced placental UCP2 expression associated with chorioamnionitis, maternal inflammation (evidenced by increased maternal TNFα levels) and SGA deliveries in preterm neonates.
O37

Effect of antenatal corticosteroids on the TGFβ-pathway and Caveolin-1 in the ovine fetal lung after LPS exposure

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2 Children’s Hospital, University of Würzburg, Würzburg, Germany
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4 Cincinnati Children’s Hospital Medical Center, University of Cincinnati, Cincinnati, Ohio, USA
*Authors contributed equally

Introduction: The cause of preterm birth can be multifactorial, of which exposure to inflammation has been given increasing attention. However, inflammation and antenatal corticosteroids, given to mothers at risk for preterm birth, inhibit alveolarization in the lung, initiating bronchopulmonary dysplasia (BPD). It is unclear how a combined exposure to inflammation and antenatal corticosteroids, which is likely a common scenario for many preterm neonates, might affect transforming growth factor (TGF)β signaling and its downstream mediators, implicated in the etiology of BPD. We hypothesized that corticosteroids would alter the effect of LPS on TGFβ expression and its downstream mediators connective tissue growth factor (CTGF) and Caveolin-1 (Cav-1) in the fetal lung.

Materials and Methods: Ovine singleton fetuses were allocated to one of six treatment groups to receive an intra-amniotic injection of lipopolysaccharide (LPS) and/or maternal betamethasone (Beta) intra-muscularly at 107 and/or 114 days gestational age (GA). Saline was used for controls. Lambs were delivered at 120 days GA (term=150 days GA). Protein levels of TGFβ1 and 2 were measured by ELISA. p-Smad2 expression and localization were determined by immunohistochemistry. CTGF and Cav-1 mRNA levels were determined by RT-PCR, protein levels by Western blot.

Results and Discussion: Free TGFβ1 and 2 and total TGFβ1 levels were unchanged after exposure to LPS and/or Beta, although total TGFβ2 did increase in animals exposed to Beta 7 days before LPS. Smad2 phosphorylation however increased 7 days after LPS exposure, indicating TGFβ signaling. Similarly, CTGF mRNA and protein levels increased 7 days after LPS exposure as Cav-1 mRNA and protein levels dropped. Beta exposure in addition to LPS prevented these changes.

Conclusion: TGFβ signaling was indeed modulated by LPS exposure, but this effect was counteracted by maternal corticosteroids. This study suggests that the intra-uterine pro-inflammatory effects can be effectively modulated by antenatal corticosteroids affecting growth-associated factors.
Fetal leptin administration during late gestation improves aspects of lung function and maturation in the fetal sheep

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²School of Animal Biology, Faculty of Natural and Agricultural Sciences, University of Western Australia, Perth, Australia.

Introduction: Preterm delivery is associated with impaired lung function and inadequate gas exchange, leading to increased morbidity and mortality perinatally. Synthetic glucocorticoids are currently administered to promote lung maturation and prevent respiratory distress in fetuses at risk of preterm delivery, but these have adverse side effects. Leptin, a hormone typically associated with energy balance, has been detected in the fetal circulation and rises with the endogenous prepartum cortisol increase in late gestation or after glucocorticoid treatment. The fetal lung possesses leptin receptors; hence leptin is suggested to play a role in maturation of the fetal lung during gestation. This study investigated the effects of fetal leptin administration on indices of pulmonary development such as lung maturation, compliance and gene expression.

Methods: Chronically-catheterised singleton sheep fetuses were infused intravenously for five days with either recombinant ovine leptin (0.5mg/kg/day, n=10; Protein Laboratories Rehovot, Israel) or saline (n=15) from 125-130 days of gestation (term ~145d) during early alveolarisation. At post-mortem (d130), lung deflation curves were performed by a manometer. Lung structure was analysed using computer assisted stereology, and gene expression by Taqman qRT-PCR (Leptin RA (all forms), Leptin RL (long form), VEGF-R2, VEGF-A, Elastin, SP-B).

Results: Fetal leptin administration did not alter fetal body size at post-mortem. Fetal leptin administration increased the relative closing pressure of the deflation limb of the pressure-volume curve compared with saline infused fetuses (P=0.042), but did not alter other indices (maximum volume at 40cmH₂O or compliance (slope of deflation curve). Gene expression of Leptin RA (P=0.031) and SP-B (P=0.015) were increased after leptin administration compared with saline infused fetuses, whilst the other genes were unaltered. Plasma leptin concentrations were increased during the 5-day leptin infusion period (P<0.05). Alveolar wall thickness (diffusion distance) was reduced with leptin administration (P=0.006).

Conclusion: Leptin administration in the fetal sheep during late gestation improves aspects of lung maturation and function and increases the expression of genes involved in leptin signalling and surfactant production. Whether the increase in expression of these genes corresponds to an increase in lung surfactant remains to be determined.

This project was funded by the BBSRC.
Statins in the newborn period mature the lungs

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Introduction: In addition to cholesterol-lowering effects, statins convey beneficial effects on cardiovascular function by increasing endothelial nitric oxide (NO) bioavailability (Kaesemeyer et al. J Am Coll Cardiol. 33(1):234, 1999). It is also possible that statins may similarly improve NO metabolism in bronchial epithelium, which may offer an avenue to treat impaired lung function, for instance in the treatment of asthma (Ahmad et al. Am J Respir Cell Mol Biol 44:531, 2011). However, whether statins are protective in the developing pulmonary system is completely unknown. We investigated whether postnatal treatment with statins altered lung maturation in the newborn rat, an established model of lung immaturity.

Methods: One male Wistar rat pup per litter received daily i.p. either saline (n=10) or pravastatin (10 mg/kg; n=9) during P1-6. At P21, fixed and frozen lung tissue was processed for indices of pulmonary maturation.

Results: Relative to controls, postnatal treatment with statins reduced the lung tissue to airspace ratio (C; 1.13 ± 0.09 vs. P; 0.83 ± 0.09, P<0.05), it increased pulmonary elastin (C; 29.74 ± 0.83 vs. P; 32.94 ± 1.14, P<0.05) and the number of secondary crests (C; 1.38 ± 0.15 vs. P; 2.18 ± 0.21, P<0.05) expressed as a percentage of total lung tissue (P<0.05), it increased the expression of pulmonary surfactant protein C (C; 0.17 ± 0.06 vs. P; 0.27 ± 0.06, P<0.05) and antioxidant glutathione peroxidase (C; 0.24 ± 0.05 vs. P; 0.29 ± 0.04) and it decreased collagen expressed as a percentage of total lung tissue (C; 11.21 ± 0.50 vs. P; 8.88 ± 0.57, P<0.05).

Conclusions: Postnatal treatment with statins promoted lung maturation in the newborn rat. Statins may offer therapeutic value in the treatment of diseases associated with lung immaturity.
Figure 1. Mean + SEM for the lung tissue to airspace ratio (A), elastin and number of secondary crests as a % of total lung tissue (B and C), pulmonary expression of surfactant protein C and glutathione peroxidase (D and E), and % collagen of total lung tissue (E) in P21 pups treated daily with i.p saline or with pravastatin (10 mg/kg) during P1-P6. *P<0.05, control vs. statin, Students t test for unpaired data.
Statins prevent detrimental effects of postnatal glucocorticoid therapy on arterial blood pressure and the kidney in rats

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Introduction: The beneficial effects of Dexamethasone (DEX) in the treatment of premature infants at risk of developing chronic lung disease (CLD) are established. However, neonatal DEX stunts growth, promotes hypertension, cardiac hypertrophy and reduces nephron number in adulthood (1-5). The mechanisms mediating unwanted side-effects of neonatal DEX are unknown, preventing targets for the improvement of therapy to maintain beneficial, but prevent detrimental, effects. We tested the hypothesis that unwanted effects of neonatal DEX are due to impaired NO bioavailability and therefore that statins, known to increase NO (6), will be protective.

Materials and Methods: One male Wistar rat pup per litter (32 litters) received i.p. either saline or DEX in tapering doses (0.5, 0.3 and 0.1 μg/g) on postnatal days (P) 1-3 in addition to either i.p. saline or pravastatin (10 mg/kg) during P1-6. At P21, arterial blood pressure was measured under urethane anaesthesia. In addition, in littermates, plasma NOx was measured and kidneys were collected, fixed in paraformaldehyde and processed for H&E and immunohistochemical staining.

Results: DEX slowed postnatal growth. Combined pravastatin with DEX treatment marginally improved postnatal growth rate from P9-14 (fractional growth rate: C=0.106±0.006; D=0.158±0.006; DP=0.176±0.006; CP=0.119±0.007 g.d⁻¹ per g starting weight; P<0.05). At P21, neonatal DEX pups had reduced plasma NOx (C=22.3±2.2 vs. D=13.1±0.9 μM, P<0.05) levels and elevated arterial blood pressure (C=60.0±2.1 vs. D=67.0±2.1 mmHg, P<0.05), reduced kidney weight (C=0.344±0.010 vs. D=0.231±0.010 mg, P<0.05) and volume (C=138.32±6.25 vs. D=105.19±9.14 mm³, P<0.05), reduced glomerular number but increased glomerular size (Fig. 1). Combined pravastatin with DEX restored arterial blood pressure (60.9±2.0 mmHg), plasma NOx (19.9±4.4 μM) and glomerular number (Fig. 1).

Conclusion: The data support the thesis that the detrimental side effects of neonatal DEX on growth, the cardiovascular system and the kidney are due to impaired NO bioavailability. Combined DEX and statin therapy may be safer than DEX alone in the treatment of CLD in premature infants.

References
2. de Vries et al. Basic Res Cardiol 2006;101: 36-42.
Mean a) Glomerular number; and b) relative glomerular size. means ± SEM, letters above the bars show statistically significant difference (P < 0.05. One-way ANOVA, Tukey's post-test)
Differential oxidative stress responses in preterm and term placenta following n-3 fatty acid administration

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Introduction: Maternal and infant nutritional supplementation with fish oils containing dietary n-3 fatty acids (EPA and DHA) appear promising in reducing adverse neonatal outcomes in preterm infants. The mechanisms contributing to this are currently unknown but they may act via a reduction in placental oxidative stress and/or inflammation. The aim of the present study was to examine the effects of DHA and EPA on oxidative stress pathways and cytokine production in placenta of term and preterm infants.

Materials and Methods: Placental explants of term (n=8) and preterm (n=8) neonates were exposed to a bacterial mimetic (LPS) with either EPA or DHA for 24 hours, and supernatants collected for measurement of oxidative stress (malondialdehyde; MDA), total anti-oxidant capacity, and the inflammatory cytokines TNFα and IL-6.

Results and discussion: In term placenta, EPA (but not DHA) treatment significantly attenuated the LPS-induced increase in MDA (p<0.01) while both EPA and DHA treatments restored the anti-oxidant capacity of the LPS exposed term placenta. In preterm placenta both EPA and DHA did not alter MDA levels from those induced by LPS. Interestingly, in preterm placenta both EPA and DHA alone promoted oxidative stress to levels equivalent with LPS. TNFα and IL-6 levels were elevated with LPS exposure (p<0.01), and were not reduced by either DHA or EPA in term or preterm tissue. This data suggests that n-3 fatty acids exert their main effects through maintenance of anti-inflammatory pathways, and do not directly inhibit inflammation or oxidative stress production.

Conclusion: Supplementing either mothers or preterm infants with fish oils containing DHA and EPA may exert unwanted adverse oxidative stress effects on preterm infants. This may depend on both the dose of EPA and DHA and gestational age, as lower doses can exert an anti-inflammatory effect while larger doses are pro-inflammatory. These findings indicate further investigation is critically required prior to the blanket supplementation of pregnant women and their infants, either term or preterm.
Session VIII – Brain, part B

Chaired by Laura Bennet & Frank van Bel
O42

Defining the risk of early brain injury or death in the very preterm newborn: Measurement of cerebral oxygen extraction during the first 24 hours of life

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Introduction: The first hours of life are characterised by low cerebral blood flow and high oxygen demand requiring elevated oxygen extraction (cFOE). Adverse alterations to this physiology result in hypoxic ischaemia predisposing the preterm newborn to brain injury in the form of intraventricular haemorrhage (IVH). No bedside monitoring techniques exist for identifying the hemodynamic antecedents of early IVH. We hypothesised that FOE would best quantify the relative adequacy of oxygen delivery and therefore the risk of early IVH or death.

Materials and Methods: Contemporaneous measurement of total internal carotid flow (Philips IE33), cerebral tissue oxygenation index (Hamamatsu NIRO 200) and arterial oxygen content (Radiometer ABL 750) in newborns ≤ 30 weeks was performed at four intervals in first 72 hrs of life. Cerebral oxygen delivery, consumption and cFOE were derived. Brain injury was assessed by sonography. An ROC curve of cFOE vs early brain injury or death was developed.

Results and Discussion: Seventy-one newborns were enrolled; mean (SD) gestational age 27(2) wks and birth weight 917 (310)g. At 14 (7) hrs the area under the curve (AUC) for cFOE and GA vs early IVH or death were no different (0.87 (0.77, 0.95) vs 0.81 (0.69, 0.92) respectively) but better than right ventricular output (0.61 (0.45, 0.78) (p=0.01). In addition cFOE displayed better discrimination at <24 vs 48 or 72 hours of age. A cFOE of 0.4 (40%) at 14 hours had a sensitivity of 70% and a specificity of 83% for poor outcome.

Conclusion: While cerebral oxygen consumption does not change in surviving, non-injured, newborns in the first 72 hours of life, early cFOE discriminates the risk of early IVH or death in preterm newborns <30 weeks gestation in the first 24 hours at least as well as gestation alone.
O43

Effect of hypothermia on periventricular leukomalacia via attenuating the cell death of oligodendrocyte precursor cells

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Introduction: Hypoxic-ischemic (H/I) insult during labor has been well known to be one of the major causes of cerebral palsy (CP). Pathologically, H/I insult mainly affect the cortex of term and late-preterm infants, and the white matter (periventricular leukomalacia (PVL)) is involved in the infants born at early-preterm, when the subcortical white matter is populated predominantly by pre-myelinating oligodendrocytes (pre-OLs). Pre-OLs have been shown to be uniquely susceptible to H/I injury. Recently, hypothermia becomes the popular and common therapy for the term and late-preterm infants with H/I encephalopathy. However, little is known about the effect of hypothermia on PVL. The aim of this study is to elucidate whether hypothermia could rescue oligodendrocyte cell death in vivo and in vitro.

Materials & Methods: Six-day-old SD rats were subjected to left common carotid artery ligation followed by 6 % oxygen for one hour. During hypoxia, the pups were maintained either at hypothermia (rectal temperature 32±1 °C) or normothermia (36±1 °C) condition. At 5 days after the insult, animals were euthanized and the brains were processed for the immunohistochemistry of myelin basic protein (MBP). In vitro, oligodendrocyte precursor cells (OPCs) and pre-OLs extracted from E20 rat fetal brains were exposed to oxygen and glucose deprivation (OGD) for 6 hours at either hypothermia (31.5°C) or normothermia (37°C) condition. After reperfusion for 24 hours, the cell viability was assessed using the stain with calcein AM and Ethidium homodimer.

Results: The loss of MBP(+) oligodendrocytes in the white matter was rescued by the hypothermia therapy. In addition, hypothermia prevented pre-OLs and OPCs from the cell death caused by OGD in the primary culture.

Conclusion: Hypothermia is suggested to attenuate H/I insult for OPCs. Elucidating its molecular mechanism could lead to develop an intrauterine preventative strategy for periventricular leukomalacia in the future.
O44

Differences in vulnerability of the developing and juvenile rat brain to TBTO demonstrated with structural MRI and functional \(^{18}\text{F}\)FDG brain microPET imaging

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Exposure of the brain during development may have long-lasting effects on health later in life. The severity of effects depends on dose, time-point and duration of exposure. Considering environmental exposure of young people, and the development of proper study designs for safety evaluation studies, it is important to learn about the vulnerability of an individual during critical phases of life. To this end we compared in rats the effects of developmental (GD8-PN10) versus juvenile exposure (PN22-PN70) to tributyltin oxide (TBTO; 8mg/kg BW), known to affect both the developing nervous- and immune systems. Structural (MRI) and functional (\(^{18}\text{F}\)FDG microPET) brain imaging was carried out at different postnatal days (PN) (MRI: PN21, 61; PET: PN18, 21, 35, 61) and the results were compared to conventional endpoints (neuropathology: (brain-weight/size) and behaviour (functional operational battery (FOB), motor activity, startle, learning/memory tasks)). Imaging was also combined to micro array gene expression/bio-informatics analysis (PD 21, 61). Effects of TBTO appeared largest in animals exposed during development rather than adolescence (juvenile exposure). The results demonstrated that changes in MRI imaging (reduced volume of regions in the anterior part of the brain) concurred with conventional neuropathology; PET changes (disturbed \(^{18}\text{F}\)FDG uptake in brain) were found in animals with impaired behaviour. Gene expression profiling confirmed that developmental exposure to TBTO affected the developing nervous system, but the immune system as well. It was concluded that research strategies including imaging technologies like MRI and \(^{18}\text{F}\)FDG microPET are animal friendly, and moreover may further improve safety evaluation protocols as well as the prediction of animal data to man.
O45

Early glutamate receptor blockade does not augment hypothermic neuroprotection of the striatum in preterm fetal sheep

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Introduction: Therapeutic hypothermia only partially improves outcome after perinatal hypoxia-ischemia, in part because of treatment delays. Combination treatment with other putative neuroprotective agents may provide synergistic benefits. We have previously shown that glutamate receptor blockade during the early recovery phase with the non-competitive NMDA receptor antagonist dizocilpine is partially neuroprotective. In the current study, we tested whether early treatment with dizocilpine can augment neuroprotection with delayed hypothermia in the basal ganglia in preterm fetal sheep after asphyxia.

Material and methods: Preterm fetal sheep (103±1 day) were exposed to asphyxia induced by 25 min of umbilical cord occlusion (UCO). 15 min post-UCO fetuses were received either vehicle or dizocilpine (2 mg/kg bolus plus 0.07 mg/kg/h i.v. for 4 h). At 5.5h after UCO fetuses were exposed to either 3 days of whole body cooling titrated to reduce core body temperature by 3°C, or maintained normothermia. Fetal brain tissue was perfusion fixed at 7 days post-UCO and immunohistochemical staining with NeuN and activated Caspase-3, and Isolectin B4 staining were used to stereologically quantify numbers of surviving neurons, apoptotic cells, and microglia respectively, in the caudate nucleus and putamen.

Results: Asphyxia was associated with severe loss of neurons in the caudate nucleus and putamen (p<0.05), with marked induction of microglia and apoptosis. Delayed hypothermia was associated with significantly improved neuronal survival (p=0.005), and reduced microglia (p=0.004), and caspase-3 positive cells (p<0.01). MK-801 infusion had no independent or interactive effect on neuronal survival, or caspase-3 induction, but was associated with a small reduction in numbers of microglia (p=0.04).

Conclusion: Delayed hypothermia was associated with moderate overall protection of the striatum, however early infusion of dizocilpine after reperfusion did not improve this hypothermic neuroprotection. However, intriguingly, it was associated with reduced microglial induction, suggesting a potential immune modulatory role.
Postnatal melatonin improves cerebrovascular function in neonates gestated under chronic hypoxia

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Reactive oxygen species play a critical role in the etiology of many diseases during the perinatal period. In fact, newborns are more prone to oxidative stress than adults, particularly during delivery. It has been proposed that at highlands or chronic hypoxia, oxidative stress is increased and therefore is one of the causes of neonatal pulmonary hypertension. Melatonin is an endogenous potent antioxidant, acting directly as a free-radical scavenger, and indirectly upregulating antioxidant pathways and downregulating pro-oxidant mechanisms. We tested the hypothesis that melatonin protects the cerebral vascular function in chronically hypoxic sheep neonates. Ten neonatal sheep gestated under chronic hypoxia were chronically instrumented at 3 days old. Between 3-10 days old, 5 received vehicle (CN, 0.5ml.kg⁻¹.d⁻¹, oral) and 5 received melatonin (MN, 1mg in 0.5ml.kg⁻¹.d⁻¹, oral). Cardiovascular recordings were performed every day in the morning. At day 11 of age, a hypoxia-hyperoxia experiment was performed to determine the cardiovascular responses to PO₂ changes. At day 12, animals were euthanized and middle cerebral arteries (MCA) were obtained for vascular reactivity and remodelling evaluation. Carotid blood flow (CBF) was similar at day 0, however, after 5 days of treatment CBF was significantly increased in MN. This matched with the hypoxia-hyperoxia experiment, where at any PO₂, the CBF was always higher in MN relative to CN (Fig 1A). MCA from MN group showed a increased response to the vasoconstrictor serotonin and the vasodilator methacholine (MetCh, Fig 1B). In contrast, the histomorphological analysis of the MCA reveal no differences between groups. Oral melatonin treatment modifies the blood flow and vascular reactivity of cerebral arteries. We speculate that these effects are directly related to the antioxidant properties and that melatonin may be a potential therapeutic tool in neurodevelopmental and cerebrovascular diseases associated with oxidative stress and chronic hypoxia.

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**Figure 1. Cerebral vascular function.** Carotid blood flow at different levels of oxygenation (A) and endothelium-dependent vasodilator response in MCA (B). Values are mean ± SEM in control (CN, white squares) and melatonin (MN, black diamonds) neonates. Significant differences (p<0.05): * vs CN.
O47

Statins prevent adverse effects of postnatal glucocorticoid therapy on the developing brain in rats

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Introduction: Postnatal glucocorticoid therapy has well established beneficial effects on pulmonary function (1,2). However, they also induce a reduction in total cerebral tissue volume in extremely low birth weight infants (3) and increase neuromotor and cognitive disabilities (4,5). The mechanisms underlying these adverse side effects are not fully understood, but oxidative stress may play a role. In addition to their cholesterol lowering effects, statins reduce oxidative stress (6). This study investigated whether combined postnatal glucocorticoid and statin therapy would diminish unwanted side effects on brain development in rats.

Methods: On postnatal days 1-3 (P1-3), male Wistar pups received i.p. injections of either dexamethasone (DEX) (0.5, 0.3, 0.1 µg/g), DEX with pravastatin (10 µg/g), saline, or saline with pravastatin. Statins were continued from P4-6. At weaning (P21) the brains were perfusion fixed and collected for subsequent stereological analysis.

Results: At weaning, relative to controls, DEX decreased significantly total brain volume, total cortical volume and total deep grey matter volume (P<0.05). White matter volume and hippocampal volume were not decreased (Fig.1). DEX also decreased the number of neurons in the cortex (22.8x10^6 ±1.05x10^6 vs.18.9x10^6±0.78x10^6), soma volume of neurons in CA1 (1205.5±32.4 µm^3 vs. 999.3±32.3 µm^3) and in the dentate gyrus (678.6±27.8 µm^3 vs. 542.3±24.4 µm^3; all P<0.05). Simultaneous treatment of pups with pravastatin and DEX restored total brain volume, cortical volume and total deep grey matter volume (Fig. 1). It also restored number of neurons in the cortex (20.4x10^6±1.14x10^6), soma volume of neurons in CA1 (1109.4±54.8 µm^3) and in the dentate gyrus (596.9±25.4 µm^3) towards control levels. Treatment with pravastatin alone had no effect on these variables.

Conclusion: Concomitant treatment of dexamethasone with statins in premature infants may be safer for the developing brain than dexamethasone alone in the treatment of chronic lung disease.

Supported by the BHF, BBSRC & Fonds Internationalisering, University Medical Centre Utrecht

References:
(4) Barrington et al. (2001) BMC Pediatr. 1:1
Figure 1. Total brain volume and volumes of deep grey matter, hippocampus, white matter and cortex at postnatal day 21 in control (Ctrl, n=8), dexamethasone (DEX, n=7), dexamethasone with pravastatin (DEX+stat, n=8), and control with pravastatin (Ctrl+stat, n=7) pups. * P<0.05 versus Ctrl; # P<0.05 versus Ctrl+stat, ~ P<0.05 versus DEX+stat (One-Way ANOVA + Student-Newman-Keuls).
O48

Melatonin as a potential neuroprotective therapy in intrauterine growth restriction

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Introduction: Oxidative stress is a key mechanism in the development of complications of intrauterine growth restriction (IUGR). An ovine model of IUGR was used to test the efficacy of maternally administered melatonin as a neuroprotective treatment in developing fetuses. This study has led to a clinical trial of oxidative stress markers in human pregnancies. Melatonin is expected to decrease levels of oxidative stress in human IUGR-pregnancies.

Materials and Methods: The pre-clinical trial consisted of 32 ewes in 4 groups (11 Control, 18 IUGR, 8 IUGR+Melatonin, 5 Melatonin-only). The first two groups received no treatment, the second two were treated using a 1g bolus of melatonin, followed by continuous infusion of 0.25mg/h melatonin iv to the ewe until natural delivery. Regular fetal blood samples were taken. Neonatal neurodevelopment was analysed using behavioural milestones, morphometric measurements and brain histology. Oxidative stress was studied in maternal and umbilical cord blood, amniotic fluid and placenta from healthy human pregnancies and IUGR-pregnancies. 8-Isoprostane and malondialdehyde, markers of lipid peroxidation were measured. Fetal ultrasound studies were obtained regularly. At postpartum, Apgar scores, birth weight and head circumference were recorded.

Results and Discussion: Blood gas analysis of IUGR-lambs indicated chronic hypoxia, which was reduced in the melatonin-group. Delayed neurodevelopment was observed in the IUGR-group, but not in the IUGR+Melatonin-group. IUGR-brains showed histological signs of neuropathology, while melatonin treatment reduced these signs. Preliminary results in umbilical cord blood and amniotic fluid from human IUGR pregnancies, show increased levels of malondialdehyde and 8-isoprostane compared to those in healthy pregnancies.

Conclusion: Melatonin is a safe, efficient antenatal neuroprotectant in IUGR. Due to its strong antioxidant effect, excellent safety profile and capacity to cross the placenta and blood-brain-barrier, melatonin is an ideal candidate for antenatal neuroprotective treatment in humans. An ethics application to treat human IUGR-pregnancies through antenatal, maternal administration of melatonin from recruitment until birth, is under review.
Melatonin protects the growth restricted fetal brain following glucocorticoid administration

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Background: Intrauterine growth restriction (IUGR) is associated with increased neurological morbidity and mortality as well as an increased risk of preterm birth. Therefore IUGR fetuses are likely to be exposed to antenatal glucocorticoids. Melatonin acts as an antioxidant and as such, may protect the fetal brain against oxidative damage.

Method: Pregnant ewes carrying twins underwent surgery at 105-110 days gestation. In one fetus we induced IUGR via single umbilical artery ligation (SUAL). Each twin was implanted with a flow probe around the carotid artery, electrocorticograph (ECoG) electrodes overlying the cortex and a femoral artery catheter. Betamethasone (BM; 11.4mg i.m. to ewe) or vehicle was given on days five (BM1) and six (BM2) following surgery. Melatonin administration (MLT; 2mg bolus, 2mg/hr i.v. to ewe) commenced 30 minutes prior to BM1. Post mortem was conducted on day seven; the fetal brain was fixed and processed for light microscopy.

Results: At 14hrs post BM1 carotid blood flow was significantly increased in both IUGR+BM (49.5±15.9% increase, p<0.001) and IUGR+BM+MLT (62.2±21.9% increase, p=0.009) fetuses, compared to pre-BM. This timepoint corresponds to an increase in the amplitude of the ECoG signal intensity in IUGR+BM fetuses (13.1±6.5% increase vs pre-BM) that does not occur in the IUGR+BM+MLT fetuses (2.2±5.5% increase). Within the fetal brain, the number of 4-HNE (lipid peroxidation) positive cells were increased in the cortex of IUGR+BM fetuses (25.9±11.2/mm²) and reduced following melatonin administration in IUGR+BM+MLT fetuses (4.9±3.7/mm²). The number of pyknotic cells were also increased in the cortex of IUGR+BM (22.2±14.3/mm²) fetuses however pyknotic cell numbers are reduced in IUGR+BM+MLT (4.0±2.5/mm²) fetuses.

Conclusions: Melatonin does not prevent the rebound carotid blood flow reperfusion that occurs in IUGR fetuses exposed to maternal betamethasone, however melatonin does prevent the increase in ECoG amplitude as well as oxidative stress and pyknosis within the fetal brain.
Session IX – DOHaD

Chaired by Emilio Herrera & Claudia Torres-Farfan
Low dose prenatal alcohol exposure induces an increase in anxiety-related behaviour but does not effect pyramidal cell number in the basolateral amygdala

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Background: Consumption of alcohol during pregnancy can be detrimental to cognitive development. Clinical studies have reported a strong correlation between prenatal alcohol exposure and the incidence of anxiety related disorders during adolescence and adulthood. Despite this, little experimental research has investigated the relationship between anxiety and prenatal alcohol exposure. The aim of this study was to investigate the effect of low dose prenatal alcohol exposure on anxiety behaviour and the basolateral amygdala (BLA) in later life.

Methods: Sprague Dawley rat dams were fed a liquid diet containing a low dose of ethanol (6% vol/vol, Ethanol n= 15) or a calorie matched control diet (Control n= 16) throughout pregnancy. Male and female offspring underwent extensive behavioural testing between 7-9 months (Adult; n= 10 male, 10 female Control; 10 male, 10 female EtOH) or 15-18 months (Aged; n= 15 male, 15 female Control; 12 male, 13 female EtOH) of age to assess anxiety-related behaviour. Subsequently, brains were collected, serially sectioned at 50µm and stained using 0.1% cresyl violet acetate. A representative volume of the BLA was calculated by measuring the area of the region over 10 serial sections. Pyramidal cell number within the BLA was assessed in male offspring (n= 4 Adult, 4 Aged per group) using the optical fractionator method for unbiased stereology.

Results: There was a significant increase in a number of measures assessing aspects of anxiety in the elevated plus maze (p<0.05), holeboard (p<0.05) and social interaction (p<0.05) tests in EtOH animals. Preliminary data found no significant difference in BLA volume or pyramidal cell number between treatment groups in male offspring.

Conclusions: Low dose prenatal alcohol exposure resulted in an increase in anxiety-related behaviour in later life that was not associated with an increase in BLA volume or pyramidal cell number.
Maternal melatonin suppression imposed by gestation under constant light has pronounced effects on global gene expression in the rat fetal heart and liver

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Introduction: This year, 10.5 million newborns will come from shift-work mothers worldwide; therefore, it is relevant to investigate whether prenatal abnormal photoperiod may program adult disease. Developmental chronodisruption is characterized by materno/fetal plasma melatonin suppression; however, it is unknown how lack of melatonin affects fetal gene expression. Here, we analysed the effects of maternal melatonin suppression on global transcription and functional genomics of the fetal heart and liver.

Methods: At day 10 of gestation, rats were randomized in three groups (n=6 each): normal photoperiod (LD), constant light (LL) and constant light with 2.0 µg/mL melatonin replacement in drinking water (LL+Mel). At 18 days of gestation maternal plasma samples were drawn for hormone assays, whereas the fetuses were subjected to biometric analysis. Total RNA isolated from fetal heart and liver was subjected to high stringency transcriptome analysis (Affymetrix array for 27,000 rat genes; SAM algorithm; FDR≤0.01; fold change +2/-2; score>2.31; P<0.05). Selected differential transcripts were validated independently. Integrated transcriptional changes were analysed using DAVID and Ingenuity Pathway Analysis.

Results: LL condition did not modify food/water intake, body weight gain nor plasma corticosterone in the dams; however, it suppressed maternal plasma melatonin and induced fetal growth retardation. Transcriptome profiling revealed changes in 229 genes in heart (134 upregulated and 95 downregulated) and 230 genes in liver (120 upregulated and 110 downregulated) of LL relative to LD fetuses. We managed to validate several selected transcripts by real-time PCR. Pathway analysis indicated modification of diverse regulatory networks by LL in both, fetal heart and liver; including cardiac steroid production, organogenesis and redox status, among several others.

Conclusion: Melatonin regulates different gene networks relevant for cardiac and hepatic fetal physiology, involving over 200 genes in each tissue. Materno/fetal melatonin suppression secondary to developmental chronodisruption may have far-reaching consequences, eventually promoting chronic disease in the offspring.

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Gender effects on renal ageing

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**Introduction:** There is a gender bias in renal function with age, with males showing considerably poorer responses. The present study considers this phenotype with reference to expression of angiotensin receptors in the kidney, and investigates the impact of gonadectomy on ageing-related changes in renal function and oxidative stress.

**Materials and Methods:** Female Wistar rats were mated and allocated to a control (18% casein) or low protein (9% casein) diet during gestation to provide a model of normal and accelerated renal ageing respectively. At 10 weeks of age, offspring were gonadectomised or exposed to a sham surgery procedure. Offspring were euthanased at 6, 12 or 18 months of age. Kidneys were collected and analysed for levels of protein carbonyls, a marker of oxidative stress. Urinary urea concentrations and creatinine clearance were assessed as markers of renal function.

**Results and Discussion:** An age and sex-related decline in renal function was observed, with differences between genders primarily attenuated by gonadectomy of males. Conversely, ovariectomy of females mediated a detrimental effect on renal function. Protein carbonyls in the kidneys of low protein offspring were significantly elevated in comparison with controls, most notably in female animals ($p<0.05$) at 18 months old. Gonadectomy of males caused a decrease in protein carbonyls ($p<0.05$).

**Conclusions:** Intact male animals appear most susceptible to renal injury, in agreement with previous work. Our work suggests there is not only a protective effect of estrogens, but also a negative impact of male sex steroids. Increases in levels of renal oxidative stress may relate to changes angiotensin receptor expression, suggesting a pathway for the mediation of gender-based differences in both normal and accelerated renal ageing.
Adrenocortical remodeling following prenatal dexamethasone treatment in a novel species – the spiny mouse

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Introduction: Antenatal stress results in elevation of maternal plasma glucocorticoids, disturbs the development of the fetal hypothalamic-pituitary-adrenal axis and steroidogenic activity of the adrenal cortex. The focus of this study was to investigate the development of the adrenal gland with respect to the synthesis and production of two important steroids - cortisol and dehydroepiandrosterone (DHEA) - in a small, rodent-like species, the spiny mouse (*Acomys cahirinus*), and to determine the effect of exposure to a brief increase of maternal glucocorticoid at mid-pregnancy on the postnatal development of the adrenal gland.

Methods: Plasma was collected from 25 days (d) gestational age (GA) to 160d after birth, and DHEA and cortisol were measured by radioimmunoassay. In separate cohorts, pregnant mice were treated with 125µg/kg of dexamethasone (DEX, n=10) or saline (n=10) at 20d GA (0.5 term). Expression of adrenal tyrosine hydroxylase (TOH), steroidogenic acute regulatory protein (StAR), 3β-hydroxysteroid dehydrogenase type 2 (3βHSD-2), 17-hydroxylase and 17-20lyase (p450c17), and cytochrome b5 (cytb5) were determined in adrenal glands at 20 weeks of age.

Results: DEX treatment did not affect adrenal structure, but expression of StAR (p=0.03), p450c17 (p=0.002), and cytb5 (p=0.001) were all significantly reduced in the zona reticularis (ZR), and at the ZR/zona fasciculata boundary; these effects were significantly more pronounced in male offspring (P<0.01). Plasma DHEA concentration was significantly decreased in offspring from DEX-treated (7.8 ± 0.6 ng/ml) vs saline-treated (12.6 ± 1.5 ng/ml; p=0.006) dams. Plasma cortisol was not affected by the DEX treatment.

Conclusion: This study shows that brief exposure to excess glucocorticoid at mid-gestation has a long-term impact on the ZR and adrenal steroidogenesis, particularly affecting synthesis and secretion of DHEA. Since DHEA is neuroprotective and important for brain development, these observations may explain some of the neurobehavioral pathologies reported to arise in children after illness and stress during pregnancy.
Impact of sex steroids on renal AT2R expression and the progression of ageing related renal injury

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Introduction: The angiotensin receptor type 2 (AT2R) promotes tissue remodelling and is overexpressed in renal injury. This effect is sex-specific and AT2R may mediate the renoprotective effects of female gender. We hypothesised that oestrogen-dependent up-regulation of AT2R ameliorates progression of age-related renal injury in developmentally-programmed hypertension.

Materials and Methods: Female Wistar rats were fed control (CON, 18%) or low protein (MLP, 9%) diet during pregnancy, with half of their offspring gonadectomised at 10 weeks old. Renal injury was assessed at 6, 12 or 18 months of age, by immunohistochemistry and measurement of albuminuria and proteinuria. Renal AT2R expression was measured by western blot.

Results and Discussion: Proteinuria and albuminuria worsened with age (P<0.001) and levels were higher in males than females across all ages (P<0.05). The latter was eliminated by male gonadectomy. At 12 months albuminuria suggested a glomerular defect in MLP offspring and by 18 months proteinuria was evident in MLP offspring. At 6 months of age, glomerular T-cell (CD3) counts tended to be higher in gonadectomised females (P=0.08). This was associated with up-regulation of renal AT2R in CON animals (P<0.05). At 12 months of age glomerular macrophage (ED1) and CD3 counts were increased in MLP females, but only if they were gonadectomised (P<0.05). At the same age, ovariectomy increased AT2R (P<0.05), which was expressed at a lower level in MLP offspring (P<0.05). At 18 months, there was a significant up-regulation of AT2R in MLP offspring (P<0.05) and this was associated with higher glomerular ED1 counts (P<0.05).

Conclusion: There is a significant impact of sex steroid exposure on the progression of ageing-related renal dysfunction and on injury in response to a prenatal MLP diet. Up-regulation of AT2R may be a compensatory response to renal injury.
O55

The rat hippocampus circadian clock: Developmental programming of spatial memory deficit by materno-fetal melatonin suppression secondary to chronodisruption

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Introduction: The hippocampus is a key structure for learning and memory processing and, at the same time, a crucial target of fetal programming. Detrimental effects of developmental chronodisruption on fetal heart and liver physiology (Spichiger et al & Abarzua-Catalan et al, FNPS-2012), led us to explore whether developmental chronodisruption -characterized by materno/fetal plasma melatonin suppression- (1) modifies the fetal hippocampus circadian clock and (2) has long-term effects on spatial memory and hippocampus circadian clock.

Methods: At 10 day of gestation (DG) pregnant rats were subjected to: constant light (LL), constant light receiving 2.0 µg/mL melatonin in drinking water (LL+Mel) or 12:12 as control photoperiod (LD). Dams were euthanized at 18-DG (every 4-h starting at 0800-h) and the fetal hippocampus was collected to measure circadian rhythms by qPCR. LD, LL and LL+Mel mothers from parallel cohorts were allowed to deliver and transferred to LD with their pups. At 90 days, males from each prenatal condition were subjected to Morris’ test to evaluate spatial memory. One-week later, they were euthanized at 1000-h and 2200-h, blood samples were collected for melatonin assay and expression of selected transcripts was measured by qPCR in the hippocampus.

Results: In the fetal hippocampus, clock genes (Bmal1 and Per2), clock-controlled genes (Mt2, Glu-4 and GCR) and NMDA receptor subunits 1B-3A-3B all displayed oscillatory expression in LD but not LL conditions. In the adult LL offspring, we observed complete lack of day/night differences in plasma melatonin as well as in hippocampus’ transcription rate of clock genes, clock-controlled genes and NMDA receptor subunits; accompanied by a significant deficit of spatial memory. Notably, maternal melatonin replacement (LL+Mel) reversed the effects observed in LL fetuses and adults.

Conclusion: Lack of melatonin during gestation is detrimental for maturation and physiology of the hippocampus, supporting that maternal melatonin is a key signal for central nervous system development.

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Postnatal β-adrenergic desensitization caused by chronic prenatal hypoxia is linked to a decreased β1/β2AR ratio and an increase in Gαs

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Despite the importance of β-adrenoceptors (βARs) in fetal development of the heart and cardiovascular regulation, little is known about how adverse prenatal events effect adult βAR function and βAR-related pathologies such as cardiac failure and hypertension. We previously showed that chronic prenatal hypoxia causes βAR desensitization in the adult chicken heart without affecting receptor abundance. Here we seek answers to what causes the observed desensitization.

We assessed cardiac tissue for β1/β2AR subtype ratio, Gαs vs. Gαi protein expression, adenylyl cyclase 5 (AC5) expression and cAMP accumulation following βAR stimulation.

Broiler eggs were incubated in normoxia (21% O2) or hypoxia (14% O2) from day 0. Hatchlings were raised to 5 weeks of age in normoxic conditions. Hearts were sampled and the β1AR/β2AR ratio in intact heart slices was assessed through competitive binding of [3H]CGP-12177 with specific β1AR or β2AR blockers (CGP-20712A and ICI-118,551 respectively). Gαs, Gαi and AC5 expression was assessed by Western blot and an immunoassay was used to determine the cAMP accumulation in isolated cardiomyocytes after βAR stimulation with βAR agonists.

There was a decrease in β1AR/β2AR ratio, increase in Gαs and no change in AC5 or Gαi expression in prenatally hypoxic hearts. The desensitization of βARs to epinephrine in 5 week chicken hearts was confirmed by significantly lower cAMP accumulation in response to βAR stimulation compared to controls. Furthermore, the β1AR/β2AR ratio in prenatally hypoxic animals was decreased similarly to the β1AR/β2AR ratio change seen in heart failure. The increase in Gαs expression is somewhat contradicting the lower cAMP accumulation in response to βAR stimulation, but the increase in Gαs may be in form of inactive protein.

In conclusion, the observed βAR desensitization in 5 week prenatally hypoxic chickens is linked to a change in β1/β2 ratio and they may be displaying early signs of heart failure.
Does concurrent growth restriction alter the effects of neonatal hyperoxia on pulmonary structure and function in adulthood?

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Introduction: Very preterm infants usually require supplemental O₂ due to lung immaturity. We have previously shown in mice that neonatal inhalation of hyperoxic gas (hyperoxia) causes alterations to the structure of the small conducting airways (bronchioles) in adulthood, but also results in restricted pup growth likely due to maternal weight loss. Our objective was to determine the effects of neonatal hyperoxia alone, in the absence of postnatal growth restriction, on lung structure and function in adulthood.

Materials and Methods: Newborn mice (C57Bl/6J) inhaled hyperoxic gas (65% O₂) from birth until postnatal day 7 (P7d), after which they were raised in room air until adulthood (P56d). During hyperoxia-exposure (H-E), dams either remained in 65% O₂ (H-E group), or were alternated between hyperoxia and room air every 24h (H-E+dam rotation (DR) group) to prevent maternal and offspring weight loss. Control dams and pups inhaled room air. At P56d, lung function was assessed, and the structure of the bronchioles and lung parenchyma was morphometrically analysed.

Results and Discussion: H-E dams were lighter than control and HE+DR dams during hyperoxia. H-E pups had reduced growth; H-E+DR pups grew at the same rate as controls. At P56d, lungs of H-E and H-E+DR pups had larger alveoli, less parenchymal tissue and more bronchiolar epithelium than controls. The bronchiolar epithelium of H-E+DR pups had increased proportions of proliferating and ciliated cells, and a decreased proportion of Clara cells. H-E pups had increased areas of airway smooth muscle and collagen, whereas collagen was reduced in H-E+DR pups. Dynamic respiratory system compliance was increased in HE+DR pups (p<0.05), and transpulmonary resistance tended to be decreased (p=0.055); H-E pups had similar lung function to controls.

Conclusion: Neonatal hyperoxia in the absence of postnatal growth restriction induces persistent structural alterations in the bronchioles and lung parenchyma, which results in lung dysfunction.
Session X – Cardiovascular, part B

Chaired by Helena Parkington & Willem de Vries
O58

Intra-amniotic lipopolysaccharide exposure induces aberrations in fetal heart development

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Introduction: Preterm birth affects 8-12% of all pregnancies and is the leading cause of neonatal morbidity and mortality. Chorioamnionitis (inflammation of the fetal membranes) is a common feature of pregnancies that end in preterm birth. Chorioamnionitis is known to affect the development of fetal organs but little is known about how it affects the development of the heart.

Materials and Methods: Using ultrasound guidance, a single intra-amniotic dose of LPS (10mg, E. coli 055:B5, n=5) was administered to ewes on day 121±1 of gestation (term is ~145 days); 5 control ewes received either a saline injection or no injection at the same time. Seven days later ewes and fetuses were euthanised and their hearts excised and perfusion-fixed. Cardiomyocyte dimensions and nuclearity (in the sheep, mononucleated cardiomyocytes are immature, binucleated are mature) were determined using confocal microscopy. Cardiomyocyte proliferation was assessed using Ki-67 immunohistochemistry. Extracellular matrix deposition in the heart was determined using picrosirius red staining followed by image analysis. Data were analysed by an unpaired t-test.

Results and Discussion: Heart weight was not different between groups (p = 0.486). In LPS-exposed fetuses, cardiomyocyte maturation was accelerated (i.e. more binucleated cardiomyocytes; p < 0.001) in the right ventricle only. In both ventricles cardiomyocytes were significantly larger (RV, p < 0.0001; LV, p < 0.0001) than in the control hearts and cardiomyocyte proliferation was markedly increased (p < 0.001). There was also a significant increase (p = 0.012) in extra-cellular matrix deposition within both ventricles of LPS-exposed fetuses.

Conclusion: Intrauterine inflammation alters cardiomyocyte growth and structure in the fetal myocardium. If the myocardial changes induced by LPS persist, they may compromise cardiac function in the neonatal period and in later postnatal life.
Smoking in pregnancy: Effects on fetal heart rate variability

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Introduction: Studies evaluating the acute effects of smoking on fetal heart rate (FHR) characteristics show conflicting results. We are making domiciliary recordings of FHR for up to 16 hours using an ultra-portable fetal electrocardiogram (ECG) device (Monica AN24). Monica software supports the extraction of average FHR, Dawes-Redman FHR parameters and monitors beat to beat variability.

Materials and Methods: Recordings from 70 smokers were compared with recordings from 70 gestational age matched controls (non-smokers). ECG electrodes were placed on the mother’s abdomen in standardised positions. Mothers who smoked were asked to press an “event” button on the AN24 whenever they smoked. The processed data raw electrophysiological data was analysed. Recordings where a continuous 90 minutes of FHR data was available, with three 30 minutes frames before, during and after smoking, were evaluated. In non-smokers the frames from matched times of the day were labelled as period 1, period 2 and period 3 respectively. Statistical analysis was undertaken by using repeated measures ANOVA. Results are expressed as mean [SD].

Results and Discussion: Fetuses exposed to cigarette smoke exhibited highly statistically significant reductions in short term variability (STV), long term variability (LTV) and beat to beat variability expressed as root mean square of successive difference (RMSSD).

Conclusion: Results are suggestive of alteration of balance between parasympathetic and sympathetic cardiac control, from a complex interplay of fetal hypoxia and/or absorption of toxins from cigarette smoke, raising potential concerns of adverse cardiovascular prognosis for the fetus and neonate.

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|c|}
\hline
\textbf{Smokers} & \textbf{Before Smoking} & \textbf{During Smoking} & \textbf{After smoking} & \textbf{P value} \\
\hline
STV (ms) & 11.5 [2.7] & 9.5 [2.4] & 10.4 [2.5] & <0.000 \\
LTV (ms) & 62.7 [16.2] & 50.7 [11.9] & 56.9 [15.0] & <0.000 \\
RMSSD (ms) & 10.7 [1.9] & 10.1 [2.1] & 9.9 [2.0] & <0.001 \\
\hline
\end{tabular}
\caption{Table 1:}
\end{table}

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|c|}
\hline
\textbf{Non-smokers} & \textbf{period 1} & \textbf{period 2} & \textbf{period 3} & \textbf{P value} \\
\hline
STV (ms) & 10.7 [3.3] & 10.9 [3.5] & 10.2 [3.5] & 0.09 \\
LTV (ms) & 57.4 [17.0] & 58.8 [18.0] & 54.9 [17.9] & 0.08 \\
RMSSD (ms) & 10.3 [2.0] & 10.4 [2.0] & 10.1 [2.1] & 0.49 \\
\hline
\end{tabular}
\caption{Table 2:}
\end{table}
Heart and coronary vessel function in normally grown and growth restricted sheep fetuses treated with melatonin

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Introduction: Intrauterine growth restriction (IUGR) predisposes to altered cardiovascular function. Clinical and experimental data demonstrate oxidative stress in IUGR, which likely mediates adverse changes in heart and coronary vessel function. Melatonin (MT) is a strong antioxidant that can safely be administered during pregnancy. We investigated the effects of antenatal MT therapy on heart and coronary function in control and IUGR sheep fetuses and lambs.

Methods: IUGR was induced surgically by single umbilical artery ligation (SUAL, or sham-SUAL for controls) at 110 days of pregnancy. We studied 2 groups of IUGR and control animals; i) short-term, with MT (2mg/h, iv to ewe) from day 5 to 7 and the fetus killed and heart collected on day 7 for assessment using a Langendorff apparatus; and ii) long-term, with MT (0.25mg/h) administered continuously to ewes carrying an IUGR or control fetus, until term birth. Lamb were killed when 48h old and coronary vessels studied.

Results: IUGR fetuses weighed significantly less than controls at both short and longer times after SUAL. IUGR resulted in an increase in cardiac contractility, a 4-fold increase in infarct area following brief ischemia/reperfusion, and an increase in coronary stiffness. MT treatment did not influence cardiac function. In both control and IUGR fetuses MT resulted in a marked increase in coronary flow in intact hearts. Isolated coronary studies revealed an increase in endothelial production of nitric oxide (NO) and dilator prostanoid by MT, and abrogation of coronary stiffness. These effects of MT occurred in both control and IUGR arteries. However, MT worsened the effectiveness of endothelium-derived hyperpolarizing factor, important in small vessels, especially when NO bioavailability is reduced.

Conclusion: IUGR programmed short-term alterations in heart function and these was not prevented by MT. IUGR also resulted in long-term coronary dysfunction and this was prevented by MT via increasing NO bioavailability.
Increased activated caspase-3 in conductive cells in the fetal heart after prolonged increase in maternal cortisol

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Introduction: We have previously found that increases in maternal cortisol in late gestation leads to enlargement of the fetal heart. Chronic infusion of pregnant ewes with cortisol from 120 to 130 days gestation resulted in a modest increase in maternal cortisol (<50%) and significant enlargement of the heart (heart weight/ body weight; 15%) or septal, right and left ventricular wall thicknesses (20%). This was associated with an increase in Ki67 staining in the heart (RV: 1.52±0.18 vs 0.95±0.09; LV: 1.66±0.19 vs 0.86±0.17 % of nuclei), and an increase in apoptosis, as measured by activated caspase-3 staining. Caspase-3 staining was marked within the subendocardial layers of the left and right ventricle, including the septum (control vs cortisol in LV: 0.027±0.008 vs 0.113±0.028; septum: 0.030±0.013 vs 0.118±0.030 % area stained).

Materials and Methods: To identify cell types expressing activated caspase-3, we co-stained for activated caspase-3 and c-kit, a stem cell marker, or periodic acid schiff (PAS). In the sheep, the large conductive Purkinjie fiber cells are localized predominantly in the subendocardial layers and are identifiable by dark PAS staining.

Results and Discussion: Analysis of sections from fetuses of control and cortisol-treated ewes indicate caspase staining in at least 4 cell types: 1) cardiomyocytes; 2) diffusely scattered c-kit positive cells; 3) cells with fibroblast morphology, localized to collagen-abundant regions between myocyte bundles or the subendocardial layer; and 4) large conductive cells with PAS-abundant staining localized in bundles within the subendocardial layer and/or projecting into the ventricular wall. In the cortisol-treated fetuses, cells with Purkinjie characteristics are the predominant caspase-3 positive cell type in the subendocardial layer.

Conclusion: Cortisol alters remodeling of the ventricular wall and the developing conduction system in the preterm fetus. These results have implications for fetal and neonatal cardiac function and survival in fetuses of chronically stressed mothers.
Videomicroscopy of neonatal microvasculature: relationship to laser doppler flowmetry

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Introduction: Our aim was to study the microvasculature of neonates with an additional tool to laser doppler flowmetry. Our hypothesis was that laser doppler baseline blood flow would be correlated with either the structure of the microvasculature and/or the blood velocity in the blood vessels as determined by videomicroscopy.

Materials and Methods: We performed videomicroscopy and laser doppler flowmetry on a cohort of 134 neonates at 6, 24 and 72h post natal age. The cohort was stratified into 3 groups based on gestational age (weeks): <29, 29-36, >36. Videomicroscopy uses sidestream, dark field orthogonal polarised microscopy (microscan) to capture a video which can be analysed for microvasculature structure and blood velocity.

Results and Discussion: Structural analysis of the microvasculature using videomicroscopy found no correlation with laser doppler baseline microvascular blood flow for any gestational group. Blood velocity analysis using videomicroscopy found no correlation with laser doppler baseline microvascular blood flow for the most vulnerable gestational group of <29 weeks. However; with increasing gestational age (29-36 weeks); we found that baseline doppler blood flow is positively correlated to the following videomicroscopy parameters: percentage of small continuous vessels (p=0.026, R²= 0.037), MFI (p=0.014, R²= 0.044), velocity of small vessels (p=0.034, R²=0.035) and flow of small vessels (p=0.028, R²=0.037).

Conclusion: In the most vulnerable gestational group of <29 weeks, there was very little correlation between microvascular structure and laser doppler baseline blood flow; suggesting that functional rather than structural measures predominate in this at risk group. The flow correlations increased with increasing gestational age in preterms; 29-36 week neonates showed a strong correlation between laser doppler baseline blood flow and small vessel blood flow as determined by videomicroscopy.
Postnatal melatonin modifies the cardiopulmonary function in high altitude newborn sheep

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Newborn lambs born at high altitude (HA) have pulmonary hypertension (PHT) and an increased pulmonary sensitivity to oxygen changes¹. Oxidative stress has a causal role in PHT, inducing pulmonary vascular dysfunction². Melatonin is an endogenous antioxidant that is relatively absent in the neonatal period³. Therefore, we hypothesized that postnatal treatment with melatonin may revert PHT.

Ten HA lambs (9-11 days old) were gestated, born, catheterized and studied at Putre (3,600 m). Five received oral melatonin (1mg in 0.5ml.kg⁻¹.d⁻¹) and five received vehicle (0.5ml.kg⁻¹.d⁻¹) for 7 days. Daily cardiovascular variables were recorded. At the end of the treatment, lambs were submitted to an experimental protocol of 5 min stepwise changes in FiO₂. Cardiac output (CO), heart rate (HR), pulmonary and systemic arterial pressures were measured and heart rate variability (HRV) was analyzed.

Hemodynamic variables were similar between groups at the beginning of treatment. After 3 days of treatment, melatonin group showed an early fall in the pulmonary arterial pressure (PAP, Fig.1A) and vascular resistance (PVR), associated with a decreased CO. In contrast, both groups showed similar cardiovascular responses to different FiO₂. However, the melatonin group showed a tendency to an increased HR, with a significantly decreased RR interval. During FiO₂ changes, melatonin treated neonates reveal a higher stability in HRV. In addition, the spectral frequency analysis showed that the melatonin group have an increased low frequency (LF), decreased high frequency (HF) and greater LH/HF ratio (Fig.1B), which implies a sympathetic predominance in the autonomic cardiac response.

The premature fall in PAP and PVR with melatonin indicates that the treatment is improving early postnatal pulmonary function. As in the perinatal life, there is a parasympathethic predominance, we speculate that melatonin is promoting early development of the cardiac function in HA neonates. Further studies will reveal if the latter effects are beneficial.


Supported by FONDECYT 1110595, 1090355 and 1080663.
Figure 1. Cardiopulmonary function. Pulmonary arterial pressure (PAP) during the treatment days (A) and the low frequency (LF) - high frequency (HF) ratio in normalized units (nu) (B) at the end of treatment. Values are mean ± SEM in control (CN, white squares) and melatonin (MN, black diamonds) neonates. Significant differences (p<0.05): † vs day 0 and * vs CN.
Session II – Poster Presentations

Chaired by Jan Derks, Joepe Kaandorp & Deodata Tijsseling
P01  Brain

Connexin 43 hemichannel blockade is not beneficial during ischaemia in the near term fetal sheep

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Introduction: Propagation of ischaemic brain injury shows a characteristic progression from severely damaged areas into previously undamaged regions. After ischaemia, connexin hemichannels open, forming a conduit between the cytoplasm and the extracellular space, resulting in cell swelling and release of ATP and glutamate. We have previously shown that post-ischaemic blockade of connexin 43 hemichannels with a specific mimetic peptide reduced seizures, improved recovery of EEG power and return of sleep state cycling and reduced loss of oligodendrocytes in the near-term fetal sheep. In the current study we aimed to determine whether connexin 43 hemichannel blockade during ischaemia, with a specific mimetic peptide, was beneficial.

Materials and Methods: Near-term fetal sheep (0.85 gestation) received 30 min of carotid artery occlusion. Mimetic peptide (50 µmol/kg/h, n=6) or vehicle (n=7) was infused into the lateral ventricle for one hour before and during ischaemia.

Results and discussion: No significant difference in impedance (cell swelling) during or after ischaemia was seen between the ischaemia only and the ischaemia–pre-infusion groups (p>0.05). No significant difference between groups was seen for recovery of EEG power, time taken for sleep state cycling to resume, spectral edge or carotid artery blood flow (p<0.05). These data suggest that connexin 43 hemichannels do not open in a consequential amount during ischaemia. Significant up-regulation of connexin 43 has been shown after ischaemia, which would allow for the opening of a large number of hemichannels.

Conclusions: These data suggest that it is unlikely that hemichannel opening is significant during ischaemia, but rather contributes to the secondary spread of injury occurring during the latent phase.
Endotoxin-mediated sensitization of the neonatal brain prior to hypoxic-ischemic insult: the role of the TNF family of pro-inflammatory cytokines TNFα, LTα and LTβ

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Perinatal brain damage is a common precursor to cerebral palsy and many other severe and permanent neurological deficits, which affects 2/1000 live births. The synergistic effect of materno-fetal infection and hypoxic-ischemic insult around the time of birth are known contributing factors.

Both in-vitro and in-vivo studies have shown that endotoxin up-regulates several molecules including signalling enzymes, adhesion molecules, chemokines and cytokines. Previous studies in our group have explored the role of the TNF family of pro-inflammatory cytokines in the damaging of the developing brain, and shown that deletion of this family cluster abolishes bacterial endotoxin sensitization to HI.

In the current study, we wanted to investigate the independent effects of TNFα, LTα and LTβ, members of the TNF cluster, and identify which one or combination of the three cytokines is responsible for this sensitization. At P7, littermate wild-type and homozygous knock-out mice for each of the three genes were subjected to hypoxic-ischemic insult. This consisted of permanent occlusion of the left carotid, followed by a 2h recovery before 30min exposure to 8% oxygen. 12h prior, a single intraperitoneal injection was administered with either 0.6µg/g LPS or saline as a control.

Pre-treatment with the endotoxin LPS resulted in a significant up-regulation in inflammation on the occluded forebrain side of WT mice (n=10) in comparison to their saline control littermates (n=10), where minimal microglial activation and cellular death was observed. Mice lacking both copies of the LTα gene showed the same non-inflammatory response when administered with saline (n=10) as their WT littermate controls (n=10). There was also a clear reduction in microglial activation and neuronal loss after LPS injection (n=10) with a significant reduction in the hippocampal and pyriform cortex regions (p<0.05) in comparison to their WT counterparts (n=10). We are currently investigating the specific effects of selective LTβ and TNFα deletions.
C-Jun plays a regulatory role in the neonatal brain's response to hypoxia-ischemia induced injury

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Hypoxia-ischemia (HI) is a major cause of neonatal brain injury resulting in cerebral palsy and other neurological disabilities. Although a number of biochemical cascades have been implicated, the downstream targets, at the level of transcriptional regulation still remain unclear. The AP1 transcription factor c-Jun is rapidly up-regulated and activated by Jun N-terminal kinase (JNK) phosphorylation following hypoxic-ischemic (HI) insult, which has suggested that this protein could serve as a master switch of the de- and re-generation program in a variety of injured neuronal and glial cells.

The aim of this study was to investigate the effects of neuronal-specific C-Jun inactivation, using cell-specific mouse mutants, on markers of neural cell damage following HI insult. HI was induced based on the Rice-Vannucci model, in postnatal day 7 mice, using 30 min (mild) or 60 min (severe) exposure to 8% Oxygen. The mice were sacrificed at 48h post HI. We used cell-specific conditional knock-out mice (n=11, 11), where C-Jun was deleted in neurons using Nestin-driven cre-recombinase in homozygous C-Jun-flox mutant mice. These were compared to littermate controls that were homozygous for C-Jun flox but did not carry the cre-recombinase (n=14, 18). Forebrain damage was assessed through extent of cell death (TUNEL), neuronal loss (Nissl) and levels of microglial activation (AlphaM&X).

Neuron-specific deletion of C-Jun resulted in a strong increase in infarct size, cell death (significance reached in striatum, p=0.04), and microglial activation in hippocampus (p=0.02), cortex (p=0.04), striatum (p=0.05), and thalamus following severe insult. A more discreet effect was also observed in the subcortical white matter with a significance increase of alphaM expression (56%). Mild insult exhibited a similar trend.

Since C-jun evidences a regulatory role in the neonatal brain’s response to injury, the use of pharmaceutical modulators of C-Jun activation could serve as a candidate target for therapeutic intervention to cerebral damage.
Pretreatment of magnesium sulphate attenuates the white matter damage by preventing the cell death of developing oligodendrocytes

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Introduction: Periventricular Leukomalacia (PVL), a lesion of the periventricular white matter, is the most common form of brain injury in early preterm infants and often results in Cerebral Palsy (CP). Some clinical trials recently showed that fetal exposure to magnesium sulfate (MgSO₄) before anticipated preterm delivery reduces the rate of the CP among survivors. However, it remains controversial that MgSO₄ has inhibitory effect against PVL. In this study, we investigated the effect of MgSO₄ against the white matter damage generated by hypoxic ischemic insult, using a modification of the Rice’s model in vivo, and the oligodendrocyte(OL) primary cultures with oxygen glucose deprivation (OGD) condition in vitro.

Materials & Methods: Unilateral ligation of the carotid artery followed by hypoxia (6% for 1 hr) in 6-day-old rats resulted in selective white matter damage in the ipsilateral hemisphere to the ligation. MgSO₄ was administered 30 min before the hypoxia intraperitoneally. At day 11, the rat infants were euthanized and the brains were isolated, fixed, and processed for immunohistochemistry. OL’s markers at each developmental stage and CD-68(+) microglia were used to assess the extent of the white matter damage. In OL primary culture, MgSO₄ was administered just before the OLs were subjected to OGD condition, and we used LDH assay to assess the cell viability.

Results & Discussion: MgSO₄ had prophylactic effect against the loss of Olig2, MAG, and MBP in the ipsilateral hemisphere to the ligation in P11 neonatal rat pups. MgSO₄ also prevented the cell death of developmental OLs which were subjected to OGD condition.

Conclusion: This study intimated that pretreatment of MgSO₄ attenuates the white matter damage by preventing the cell death of developing OLs.
Fetal programming of left ventricle’s morphology and day/night gene expression by maternal chronodisruption

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Introduction: Maternal photoperiod is transduced into a fetal internal temporal order through different signals, including melatonin—a strong circadian synchronizer. Already at 18 days of gestation (DG), several rat fetal organs display circadian rhythms. However, the integrated short- and long-term consequences of materno/fetal circadian crosstalk disruption are unknown. Here, we explored the effects of developmental chronodisruption (associated with maternal melatonin suppression) on fetal and adult cardiac circadian rhythms and adult heart morphology.

Methods: At 10-DG pregnant rats were subjected to: constant light (LL), constant light receiving a daily dose of melatonin (LL+Mel) or 12:12 as control photoperiod (LD). Mothers were euthanized at 18-DG (every 4-h starting at 0800) and the fetal hearts were collected to measure circadian rhythms by qPCR. LD, LL and LL+Mel mothers from parallel cohorts were allowed to deliver and transferred to LD with their pups. At 90 days old, males gestated in each condition were euthanized, the heart was dissected out and morphology/histology and circadian rhythms (qPCR) were analyzed.

Results: At 18-DG Bmal1 and Per2 (clock genes) as well as Pgc1α, Pparα, Mt2 and Glut1-4 (clock-controlled genes) displayed an oscillatory expression in LD but not in LL condition. In adult LL offspring, we found reduced plasma melatonin concentration and no day/night oscillation of clock gene expression. Besides, adult LL hearts had increased left ventricle thickness and larger cardiomyocytes. All effects observed in fetal and adult LL hearts were prevented by melatonin replacement to values close to LD.

Conclusions: The fetal heart contains a circadian clock, which is disrupted in pregnancy under constant light. This effect carries on into adulthood and, interestingly, it is accompanied by low levels and absence of a circadian rhythm of plasma melatonin. The marked changes found in overall left ventricle morphology and also cardiomyocyte size may be associated with increased risk of cardiovascular disease.

References

Melatonin improves pulmonary vascular reactivity and histomorphometry in pulmonary hypertensive newborn sheep

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Newborn lambs born at high altitude (HA) have an increased pulmonary vascular reactivity and pulmonary hypertension (PHT). At HA, biological organisms generate a large amount of reactive oxygen species, inducing oxidative stress which is associated with PHT. Melatonin is an important endogenous antioxidant and is relatively absent in the neonatal period. Therefore, postnatal treatment with melatonin may improve pulmonary vascular function.

Ten HA lambs were gestated, born and studied at Putre (3,600 m.a.s.l.). At 3 days old, five received oral melatonin (1mg in 0.5ml.kg–1.d–1) and five received vehicle (0.5ml.kg–1.d–1) for 7 days. At day 8 of treatment, the lambs were euthanized (thiopentone, 100 mg.kg–1) and small resistance pulmonary arteries were mounted in a wire myograph to assess vascular reactivity. Perfusion-fixed lungs (formaldehyde 4%) were processed with Van-Gieson staining for histological analysis.

Control neonates have similar vascular responses as reported previously. Melatonin treatment decreased contractile capacity (K+) and increased responses to thromboxane mimetic (U46619) and serotonin (p<0.05). In addition, melatonin treated neonates enhanced relaxation mediated by NO (SNP) and melatonin. Further, endothelial vasodilator function, expressed as sensitivity (pD2) to methacholine was enhanced in melatonin treated lambs (Fig 1A). Internal and external vessel diameter, and area and thickness of smooth muscle pulmonary arteries were similar in both groups. However, the % of elastic layer relative to vessel wall (as remodeling index) was significantly decreased in neonates treated with melatonin (Fig 1B).

Postnatal melatonin improved ex vivo vascular function and decreased vascular remodeling processes in pulmonary arteries from HA neonates. We speculate that these beneficial effects are directly related to the melatonin antioxidant capacity. Melatonin may be a potential therapeutic tool in cardiopulmonary diseases associated with gestation and delivery at high altitude.

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Effects of chronic prenatal hypoxia on the chick embryo heart

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Introduction: Prenatal hypoxia causes growth restriction of the embryo, results in smaller perinatal heart size and increases the risk of cardiovascular disease in adulthood. However the effects of hypoxia on the prenatal heart growth mechanisms are less understood. Our objective was to study prenatal growth of the hypoxic chick heart to answer how cell density, mitotic activity and structural composition are affected by hypoxia.

Materials and Methods: Embryos from eggs incubated in normoxia (21% O₂) or chronic hypoxia (14% O₂) from day 0 were sampled at E15 and E19. Each egg was injected with 250µg EdU 8h prior to sampling. Embryos and hearts were weighed. Normalized wall thickness measurements were obtained from formalin fixed, sectioned hearts. Total protein and DNA were quantified from ventricular homogenates and DNA/protein ratios were calculated. Cell density in DAPI-labelled sections, extracellular collagen in antibody labelled sections and mitotic cell density in EdU-labelled sections were quantified by confocal microscopy.

Results and Discussion: Hypoxic embryos were smaller than normoxic at E15 and E19. The heart weight/embryo weight ratio was higher in hypoxic than in normoxic embryos at both E15 and E19. At E19 hypoxic embryos also had smaller hearts than normoxic embryos. Cell density, DNA/total protein content and extracellular collagen content were not different between the groups. Mitotic activity was not different between hypoxic and normoxic hearts but decreased between E15 and E19. Normalized right and left ventricular wall thickness was not affected by hypoxia.

Conclusion: Hypoxia affects heart growth differentially during development of the chick embryo but does not have any effect on mitotic activity, cell density or structural composition.
Age decreasing effect on both PGC-1α gene methylation and expression in hypertensive FHH and normotensive WKY rats

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Introduction: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) is an essential cofactor for the activity of peroxisome proliferator-activated receptors (PPARs), which are ligand-activated transcription factors involved in regulation of lipid and glucose metabolism, mitochondrial biogenesis as well as cardiac function and blood pressure. The aim of this study was to analyze the effect of age on renal PGC-1α DNA methylation and gene expression in Fawn-hooded hypertensive (FHH) rats. Age effects on renal PGC-1α gene expression were also measured in normotensive Wistar Kyoto (WKY) rats.

Materials and Methods: PGC-1α methylation was quantified at 8 CpG sites in 2 assays by Pyrosequencing in kidneys obtained from 2 day old, 2 week old and adult hypertensive FHH rats of both genders. Renal PGC-1α gene expression was evaluated through qPCR for FHH and normotensive WKY rats.

Results and Discussion: Renal PGC-1α methylation decreased significantly with age at 7 of the 8 CpG sites studied in both genders of FHH (Fig. 1 and 2). However, unexpectedly, PGC-1α gene expression decreased with age (Fig. 3). PGC-1α gene expression also decreased with age in normotensive WKY rats, where PGC-1α gene expression was ~8 times higher compared with age and gender matched FHH (Fig. 3).

Conclusion: Renal PGC-1α gene expression reduced with age in both hypertensive and normotensive rats. However, in FHH rats, PGC-1α gene methylation also decreased with age at the CpG sites investigated. These apparently discrepant results need further research.
Figure 1. Renal PGC-1α methylation in FHH with age (Assay 1).

Figure 2. Renal PGC-1α methylation in FHH with age (Assay 2).

Figure 3. Renal PGC-1α gene expression in FHH and WKY rats.

# P<0.001 versus male age-matched FHH.

* P<0.001 versus female age-matched FHH.

Normalized to 2d FHH for each gender.
Is there a critical time period for neurovegetative development in the fetus?

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Introduction: Regulation of the fetal heart rate pattern (fHRP) reflecting the development of the autonomic nervous system (ANS) can be assessed by fetal beat-to-beat heart rate variability (fHRV). The aim of this study is to elucidate evidence on the question of a critical developmental window between 28-32 wks GA.

Materials and Methods: 42 healthy singletons were studied longitudinally on 2-3 occasions during the late 2nd and 3rd trimesters by fetal magnetocardiography (fMCG) over periods of 30 minutes. The time series of fetal QRS complexes were drawn to reconstruct the fHRP and to perform fHRV. fHRP were pre-classified into quiet (fHRPI) and active patterns (fHRPII). fHRP was underrepresented in the sample. In unselected time series (30 min) and segments of fHRPII (10 min) fHRV was performed: mHR, SDNN, RMSSD, fVLF, fLF, fHF, ratio fVLF/fHF, AIF_NN, AIF_fHF, AIF_fLF and AIF_f125 (autonomic information flow). Developmental steps were intraindividually studied between 4 gestational segments (GS): (a)23/1-28/0; (b)28/1–31/0; (c)31/1-35/0; (d)>35/0 wks GA.

Results and Discussion: Particularly considering fHRPII, short term parameters significantly increase first (GS a-b) accompanied by a drop in AIF. Overall/long-term fHRV parameters are following at GS a-c. There are no significant changes between GS b-c. Beyond that GS, there are trends for increase both in the overall and short term parameter sets irrespective of the fHRP. At GS c-d a gain in fHF is accompanied by increasing AIF_fHF. A decrease in AIF_NN is lost in association with another increase in SDNN during fHRPII.

Conclusion: Different rhythms of regulation maturate at a different pace. The observed results elucidate separate phenomenons: increasing complexity of ANS regulation at (GS a-b), an emphasis on sympathetic activity near term during periods of fetal activity, development of fetal respiratory sinus arrhythmia and increasing cardiac self-regulation reducing beat-to-beat predictability that is overrun by sympathetic drive.
Intrauterine synthetic steroid exposure results in a prolonged suppression of heart rate regulation in the immature fetus

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Introduction: Intrauterine exposure to synthetic steroids (GC) results in an acute suppression of sympathetic heart rate regulation in fetuses between 29-34 wks GA [1]. We hypothesize that prolonged and gestational age dependent influences on the fetal autonomic nervous system can be elucidated by fetal beta-to-beat heart rate variability in ongoing pregnancies after GC exposure.

Materials and Methods: 29 otherwise healthy fetuses were studied by fetal magnetocardiography (fMCG) over 30 min on average 1 week (5-11 days) after GC exposure after successful management of threatened preterm labor. The sample was matched to 76 non-exposed controls and grouped by developmental age (GC1 24/0-29/0; GC2 29/1-32/0, GC3 32/1-35/0). The time series of fetal QRS complexes were drawn to reconstruct the fHRP and to perform fHRV. fHRP were pre-classified into quiet (fHRPI) and active patterns (fHRPII). fHRV was performed: mHR, SDNN, RMSSD, fVLF, fHF [2]. The interval from exposure was categorized by 5-7 vs. 8-11 days.

Results and Discussion: GC1 was characterized by a significant suppression of fHRV during fHRPII while mHR was increased. The effect disappeared >7 days from exposure. In GC2 we observed selective reduction of short term fHRV during fHRPI. No differences were found within GC3.

Most previous human studies on GC exposed fetuses observed reversibility of the side effects on cardiovascular regulation by 96 hours.

Conclusion: Our data suggest that while older fetuses display a more pronounced acute reaction [3] there seems to be a more prolonged autonomic suppression during earlier gestational ages.

References:
Fetal heart rate abnormalities in gastroschisis: a case-series and review of literature


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Objective: To study the prevalence of cardiotocographic (CTG) abnormalities and poor perinatal outcome in pregnancies complicated by gastroschisis through a case series and a review of literature.

Materials and Methods: Gastroschisis cases, born between January 2001 and September 2010 at the Department of Perinatology, University Medical Center Utrecht, The Netherlands, were studied. CTG's were reviewed and scored using STAN clinical guidelines. These results were compared with gastroschisis cases described in literature and numbers reported for the general population. Articles and cases were analyzed for cardiotocographic monitoring, asphyxia, emergency Caesarean Section for suspected fetal distress, umbilical artery pH, Apgar scores at 5 minutes postpartum and third trimester intrauterine fetal death (IUFD).

Results: Twenty-five cases of gastroschisis from our department were analyzed. Cardiotocographic abnormalities were present in 10 cases (47.6 %) and there was one case of asphyxia (4.0 %). Reduced baseline variability was the most common CTG abnormality (70 % of our cases) and occurred frequently in combination with tachycardia and decelerations. No correlation between CTG abnormalities and poor perinatal outcome could be found. Review of literature showed 27 studies describing cardiotocographic abnormalities or perinatal outcome in gastroschisis. Prevalence of cardiotocographic abnormalities was 49.8% in gastroschisis, compared to 14.1 % in the general population (P <0.0001). Prevalence of umbilical artery pH <7.05 was 1.2 % versus 2.1% in the general population (NS), with no significant difference in cases of asphyxia, 3.0 % versus 2.5 %, respectively.

Conclusion: Cardiotocographic monitoring has been advised to prevent IUFD which occurs more frequently in gastroschisis fetuses. Cardiotocographic abnormalities occur significantly more often in gastroschisis cases than in the general population. On the other hand, there were no significant differences in perinatal outcome, measured by cases of asphyxia and umbilical cord pH. This suggests that there might be another mechanism involved in the aetiology of abnormal fetal heart rate patterns.
High fat programming of beta cell hypertrophy, hyperglycemia, hyperinsulinemia, insulin resistance and hyperleptinemia in 3 month old Wistar rat offspring

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Introduction: High fat programming, by exposure to a high saturated fat diet in utero and/or during lactation, compromised beta cell development and function in neonatal and weanling rats. The effects of maintenance on a high fat diet (40% fat as energy) in utero and/or during postnatal life were therefore investigated on weight, leptinemia, glycemia, insulinemia, HOMA, lipidemia and islet morphology in 3 month old rats.

Materials and Methods: The offspring studied were HFG (maintained on a high fat diet throughout gestation), HFP (high fat diet maintenance from birth to 3 months of age) and HFGP (high fat diet maintenance throughout fetal and postnatal life). Control rats were maintained on a standard laboratory diet (10% fat as energy). Body weight, fasting circulating leptin, glucose, insulin, HOMA, triglyceride and cholesterol concentrations were determined. Pancreata were double immunolabeled for insulin and glucagon to assess beta and alpha cell number, size and volume. Islet and acinar cell proliferation were also determined. Data (mean ± SEM) were analysed with One-way ANOVA followed by Bonferroni’s post-test.

Results and Discussion: HFP rats were hyperglycemic (11.43 ± 1.50 mmol/l vs. 6.16 ± 0.47 mmol/l in control rats), hyperinsulinemic (441.90 ± 81.14 vs. 95.26 ± 21.87 µU/ml in control rats), hyperleptinemic (8.5 fold increase) and insulin resistant displaying beta cell hypertrophy and increased islet proliferation indices (0.0128 ± 0.0030 vs. 0.0032 ± 0.0006 in control rats). HFGP rats were 57% heavier, hyperleptinemic (14 fold higher) and insulin resistant compared to control rats.

Conclusion: Postnatal high fat programming disrupts islet architecture and glucose homeostasis. Specifically, postnatal high fat programming induced beta cell hypertrophy and increased islet cell proliferation concomitant with hyperglycemia and hyperinsulinemia reflecting partial beta cell compensation. These findings emphasise the importance of maternal nutrition on offspring health outcomes and further confirm the durable effects of programming.
Maternal chemical exposure forms basis for persistent neural impairment in young and adult offspring. A Rat Model with MAM

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An example of the Fetal Origin of Adult Health and Disease hypothesis (FOoAHaD) is the western Pacific amyotrophic lateral sclerosis (ALS) and Parkinson-dementia complex (PDC). Researchers propose that chemicals in cycad seed kernels, in particular the proliferation inhibitor methylazoxymethanol (MAM), induce the “selfpropelling” (progressive) western Pacific amyotrophic lateral sclerosis (ALS) and Parkinson-dementia complex (PDC). They advocate that neurodegeneration and cancer in cycling or non cycling cells such as neurons may be associated with similar pathways \cite{1}. Here, we re-evaluate effects induced by prenatal exposure to MAM in light of this hypothesis.

Mated female rats (14 rats/dose group) were dosed ip with MAM (gestation day (GD) 13–15; 0, 1.25, 2.5, 5 or 7.5 mg MAM/kg bw/day). Neuropathology survey (n=10 litters/dose; 1 animal/sex/litter) included: gross abnormalities, brain-weight, microscopic slide reading, linear morphometry (PN22, PN62) and stereology (PN22: neurons numbers hippocampus; cerebellum) \cite{2,3}. Motor activity was on PN13, 17, 21 (61) \cite{4,5}.

Microcephaly, observed in cerebrum but not cerebellum (7.5 and 5mg/kg MAM groups (PN22, PN61)), was supported by dose-related reduction in brain weight, brain-layer-width in frontal/parietal cortex and hippocampus –areas in the proliferation phase during exposure to MAM, and involved in spatial exploration\cite{6}– and neuron reduction (7.5 mg MAM/kg group) in hippocampus and not the cerebellum. No brain tumors or gross abnormalities in other organs were found. Motor activity showed hypo-activity on PN13, hyper-activity on PN17 and 21; no differences on PN61, suggesting a developmental delay rather than permanent locomotor impairment \cite{2,3,4,5}.

Our effects are explained by a temporarily arrest of cell-proliferation during the fetal MAM-exposure window with no further effects afterwards. We were not able to support the hypothesis of self-propelling progressive neurotoxicity. However, these experiments evidently show that prenatal exposure to low doses of environmental chemicals can have long-lasting effects on health and disease in later life.

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Gender differences in the development of physiological variables in postnatal lambs from birth up to 1 year of age

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Background: There is increasing evidence on the importance of gender differences in cardiovascular and metabolic syndrome (e.g: diabetes, obesity, stroke) as well as depression. In the era of personalized medicine, understanding about these gender differences is essential for both diagnosis and management. Therefore, in this study, using sheep, we investigated the gender and age effects on the development of variety of physiological variables in the postnatal lambs from ~ postnatal day 3 to 1 year-old.

Methods: Arterial pressure, heart rate, ECG, arterial blood gases, pH, glucose, lactate, ACTH, cortisol and urine volume were measured at 3, 10 day; 1, 3, 6 and 12 months. Sleep-activity cycles were measured using an Actiwatch around the neck of the lambs. For activity data, the area under the total activity curve versus postnatal age for the first 90 postnatal days was calculated for each lamb. The effects of gender and age on cardiovascular, metabolic and endocrine function and effects of gender and lamb number on activity variables in the postnatal lambs were determined using 2 way repeated measure ANOVA following by Bonferroni’s correction. Piecewise linear regression analysis with 2 elements was performed to determine changes in the variables with age.

Results: Gender differences in heart rate, LF power and pH were observed at 6 months (p=0.016) and 3 days of age (p=0.036; p<0.001), respectively. Among the variables, the break points for significant changes of cortisol, Hb, urine flow rate, SDNN, heart rate and glucose values were at 0.51 ±0.09, 1.49 ±0.23, 4.56 ±0.7, 5.31 ±1.1, 5.59 ±0.5, 7.44 ±1.4 weeks of age, respectively.

Conclusions: Our results showed most of the physiological variables occur around 1 month of age which is most likely due to the time of weaning. Gender differences in cardiovascular and metabolic functions were also found, similar to human findings.

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The Andean curse on the conquistadors: A comprehensive study of high altitude hypoxia and birth weight in 24,827 babies

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Introduction: Investigations relating low birth weight with increased rates of cardiometabolic diseases in adult life have focussed on human populations undergoing maternal undernutrition or famine during pregnancy1,2. Over 140 million people live at altitudes higher than 3000 m, comprising the largest single human group at risk for fetal growth restriction due to the uniform factor of hypoxia. However, no study has related high altitude-induced alterations in birth size to later diseases. We have created a cohort of 24,827 human birth records in Bolivia to serve as a platform to investigate such relationships. In this study, we determined in this cohort: 1) the effects of high altitude hypoxia on birth size and 2) whether high altitude native ancestry or female gender conferred protection.

Methods: Using a multi-institutional approach, records from deliveries between 1975-1985 were obtained over two years from obstetric clinics and hospitals in the cities of La Paz (3600 m) and Santa Cruz (420 m). Only singleton, non-smoking healthy pregnancies that reached term (>37 weeks) were assessed. Ancestry was determined by validated analysis of paternal and maternal surnames3.

Results: High-altitude pregnancy reduced body weight and length at birth. High-altitude native ancestry conferred protection against effects on birth weight but not on length (Fig. 1). Consequently, babies born at high altitude of European ancestry were the thinnest for their length at birth. The effects of high-altitude pregnancy on birth size were similar among male and female babies.

Conclusions: This comprehensive cohort of Bolivian birth records shows that high-altitude native ancestry but not female gender protects against the effects of high-altitude pregnancy on birth size. Babies born at high altitude of European ancestry may be the most susceptible to increased risk of disease in later life.

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References
Figure 1. Effect of pregnancy at high altitude on birth size by ancestry. Values are mean+SEM for birth weight (a) and birth length (b) for babies born at low and high altitude from Andean (■), Mestizo (grey square) and European (□) ancestry. Different letters are significantly different, GLM ANOVA (P<0.05).
Risk-adjusted cesarean delivery rate as obstetric clinical indicator

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Introduction: As high risk pregnant patients are prone to deliver by cesarean section, overall cesarean delivery rate is not a good clinical indicator in obstetrics. We examined whether risk-adjusted cesarean delivery rate is appropriate as obstetric clinical indicator using risk scoring system for pregnant woman that is fairly standard in Japan.

Materials and Methods: The risk scoring system was proposed for self-assessment of pregnant woman to choose the facility for delivery, i.e. small private facility for low risk women and fetal-maternal medical center for high risk women. Score1 is for mild risk, 2 for moderate risk and 5 for high risk. There are 18 items in the early pregnancy and 11 items later in pregnancy. Total score of 0 or 1 is considered to be at low risk, 2 or 3 at medium risk and more than 4 at high risk. We applied this score to the singleton pregnant woman delivered in our hospital between 2009 and 2011 and examined cesarean rate for low risk women.

Results and Discussion: Out of 2900 deliveries, 31.6% were low risk. Cesarean rate for low risk women was 11.0% in 2009, decreased to 8.9% in 2010 and increased to 10.7% in 2011. In 2011, cesarean rate was 6.8% before the Great East Japan Disaster on March 11 and 11.5% after the disaster. Among the evacuees, the rate was 17.7% while 11.1% for non-evacuees. Although these changes were not significant, this indicator may be useful to review and improve the clinical practice.

Conclusion: Risk-adjusted cesarean delivery rate by pregnancy risk scoring can be used as obstetric clinical indicator to review and improve the clinical practice.
Infant attachment: The contribution of infant functioning and mother-infant interaction

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Objective: to assess the effect of infant’s psychophysiological functioning early in life on the quality of mother-infant interaction and on later attachment. To explore the mediation effect of the quality of mother-infant interaction on the association between the infant’s psychophysiological functioning and attachment security.

Method: a longitudinal prospective design was conducted with 94 infants and their mothers. Eight weeks-old infants were assessed with the Neonatal Behavioral Assessment Scale and the Alarm Distress Baby Scale. At 8 to 12 weeks old, cortisol levels were measured both before and after routine inoculation. Mother-infant interaction was evaluated at 12-16 weeks, using the Global Rating Scales. The strange situation procedure was performed at 12 months.

Results: The overall quality of mother-infant interaction mediates the relation between infant’s psychophysiological profile and infant attachment.

Conclusion: the co-construction of the mother-infant relationship has bidirectional origins on the infant characteristics and on patterns of interaction.
Maternal anxiety in early pregnancy is associated with infant sensory-cognitive development: an event-related brain potential study in 2-month olds

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Introduction: Processes studied under the Developmental origins of Behavior, Health and Disease (DOBHaD)-concept encompass variations in both typical and atypical developmental and maturational patterns which are seen as adaptation to the environment resulting from gene-environment interaction. The present study tested in 2-month-old infants whether prenatal exposure to maternal anxiety affected event-related brain potentials (ERP) to rapidly presented sound sequences, possibly reflecting alterations in content and strength of sensory-cognitive processes.

Materials and Methods: Maternal State Anxiety (N=71) was measured with the State Trait Anxiety Inventory at 8-15 weeks of pregnancy (M = 33; SD = 9). Infants ERPs were measured during a passive oddball paradigm delivering 4 types of sound events of 200ms and an interstimulus interval of 300ms, i.e: (1) 1500 frequent standard complex tones of 500Hz, (2) 150 tones with a deviant 100ms pre-stimulus, (3) 150 deviant white noise segments, (4) 150 deviant environmental sounds.

Results and Discussion: (1) A multivariate repeated measures ANCOVA, performed on the 220-280 difference wave with State Anxiety as a continuous predictor and behavioural state (‘awake’ versus ‘asleep= REM-sleep and non-REM sleep’) as a covariate revealed a main effect for prenatal anxiety on infant Mismatch Negativity Response, a deviance detection ERP-component (p<.05); (2) Repeated measures ANCOVA’s with State Anxiety scores as predictor variable, Electrode Position (F3,Fz,F4,C3,Cz,C4,P3,Pz,P4) as within-subjects factor and behavioural state as between-subjects factor revealed a main effect of State Anxiety on amplitude as well as on peak latency of the standard sound (both p’s < .05).

Conclusion: Our preliminary analyses indicate that fetal exposure to maternal anxiety in 1st trimester: (1) may alter sensory-cognitive processing involved in detecting spectral and temporal deviance in sound features (2) may increase ERP responsivity. Our follow-up study will empirically verify whether infant altered sensory-cognitive processing may result in poor emotional and stress regulation in toddlerhood.
Rhythmic embryonic movements in humans and guinea pigs

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Introduction: Sideways bending (SB) of head and/or rump, the earliest embryonic motility in both humans and guinea pigs, can be visualized by means of ultrasonography.(1,2) We evaluated if the early embryonic motility is rhythmic as in other species. The distribution of SB-to-SB intervals was explored in human and guinea pig embryos prior to the appearance of more complex movements such as general movements. We hypothesized that the activity in both species is cyclic.

Materials and Methods: From 15-min sonographic recordings of SBs between 5 weeks and 0 days (5wk0d) and 7wk0d conceptional age (CA) in 18 human embryos of uncomplicated IVF pregnancies (term 38 weeks) and in 20 guinea pig embryos between 3wk4d and 4wk0d CA (term 9 weeks), SB-to-SB interval durations were categorized as long (≥10 s) or short (<10 s) intervals.

Results and Discussion: For human embryos, the median values for long and short intervals were 61 s (range, 10–165 s) and 3 s (range, 1–9 s) respectively; for guinea pigs 38 s (range, 10–288 s) and 5 s (range, 1–9 s) respectively. During development, the duration of long intervals decreased while the number of short intervals increased for both species. The earliest embryonic motility in the human and guinea pig is performed rhythmically with distinct developmental milestones.

Conclusion: Motility assessment before birth can be used to evaluate the integrity of the nervous system. The resemblance of their interval development offers promising possibilities to use the guinea pig as a non-invasive animal model of external influences on motor and neural development.

References:
Fetal Alcohol Syndrome in a rat model: brain $^{18}$FFDG microPET and facial anomalies

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Following excessive maternal consumption during pregnancy, descendants are at high teratogenic risk to develop fetal alcohol syndrome (FAS). Children with FAS show facial anomalies, delayed development, neuro-cognitive and behavioural disabilities. Questions addressed were: 1) Does a rat model of fetal ethanol exposure show similarities with humans when it comes to facial anomalies? 2) Can we derive and quantify these facial anomalies from photographs taken straightforward from the rat’s snout, similar to the diagnosis methods applied in humans? 3) Are there differences in $^{18}$FFDG uptake between different brain regions 4) Can we relate these differences to those found in craniofacial anomalies? An Extended One Generation Reproduction Toxicology study was performed in rats [Guideline OECD433]. From 2 weeks pre-mating to postnatal day (PN) 70 of the offspring, rats were daily exposed to different ethanol concentrations via the drinking water (0, 1.5, 4.0, 6.5, 9.0, 11.5 or 14%; highest dose on water from PN21 onwards). On PN18,21,35,61 the offspring’s brain activity was measured using $^{18}$FFDG microPET imaging. Snouts of offspring were photographed with a high resolution camera on PN8,37,61,70. In concordance with the analysis in humans, a number of parameters was selected including height of the philtrum, width and height of nose. Ultrasonic vocalisations were monitored (PN4-18) and conventional daily testing of body weight, senses and reflexes was performed. The development of ethanol exposed animals appeared delayed and in some behavioural tests persistent impairment in the adult animal is observed. Brain activity is reduced in many brain areas, but the regions in the back of the brain are more affected than those in the front of the brain. The results of the brain activity measured by $^{18}$FFDG uptake, and the facial anomalies suggest that midline brain region anomalies might be underlying to the (recovery from) craniofacial anomalies during the postnatal period.

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Thymic involution and placental features in SGA fetuses: a preliminary report

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Introduction: We recently reported that thymic volume (TV) is reduced in small for gestational age (SGA) compared with appropriate for gestational age (AGA) fetuses, and in IUGR due to placental causes compared with constitutional SGA. We hypothesized that a specific “trigger” (subclinical infection) could compromise trophoblastic invasion and thymic development. The purpose of this study was to identify placental histological signs of infections in SGA fetuses with thymic involution.

Methods: We revised histological exams of 27 placentas from SGA fetuses (fetal abdominal circumference (AC) and birthweight <10th percentile for GA). We defined constitutional SGA those fetuses with normal uterine (UAD) and umbilical artery Doppler (uaD), while IUGR were those with abnormal UAD, further divided based on uaD (normal or abnormal uaD). For each fetus, histological signs of infection/inflammation were recorded and TV was reconstructed. To normalize the TV for the size of the fetus, the TV/AC ratio was calculated.

Results: There were no infective agents isolated from placentas. Signs of inflammation were found in 14/27 cases (52%): 6/27 (22%) were chronic villitis. IUGR with abnormal uaD presented a higher incidence of chronic villitis (5/14 43%). These fetuses also presented the lowest TV/AC ratio compared to both constitutional SGA (n=4) and IUGR with normal uaD (n=9).

Conclusions: In our population, we didn’t identify any infectious agents but we reported a high incidence of chronic villitis of unknown origin (VUE) in association with a reduced thymic volume. In literature, VUE ranges from 12 to 86% in IUGR and 5-11% in AGA babies. It is still debated if VUE is a response to infections or represents a type of host-versus-graft reaction. The relationship between thymic involution and VUE in SGA and IUGR fetuses could lend support to the hypothesis of a subclinical infection, but needs to be tested with more studies.

References:
The breadth of the placenta is associated with body size at birth

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**Introduction:** Studies of pregnancies complicated by preeclampsia led to the suggestion that the surface of the placenta is aligned along two axes, measured by its breadth and length. It was hypothesised that tissue along the breadth serves as a nutrient sensor, responding to the mother’s nutritional state and fetal nutritional demands, while tissue along the length has different functions.

**Methods:** We measured the breadth and length of the placental surface in 401 neonates born in the King Khalid Hospital, Riyadh, Saudi Arabia, and related these measurements to the baby’s body size.

**Results:** The breadth and length of the placental surface were highly correlated (coefficient=0.7). Nevertheless, in a simultaneous regression with both measurements only the breadth was associated with neonatal body size. There were strong trends of increasing birth weight, ponderal index, and the circumferences of the head, chest, abdomen and thigh with increasing placental breadth. In contrast no measurement of baby’s body size was related to placental length. Birth weight increased by 125g per cm increase in placental breadth (95% confidence interval 88 to 162, \(p<0.001\)) but only by 20g per cm increase in placental length (-13 to 53, \(p=0.2\)). The corresponding figures for head circumference were 0.28cm (0.17 to 0.39, \(p<0.001\)) and 0.03 (-0.07 to 0.14, \(p=0.5\)). The associations between placental breadth and neonatal body size were strongest if the mother’s height was below the median (157cm).

**Conclusion:** The associations between a larger breadth of the placental surface and a larger baby are consistent with the hypothesis that tissue along the breadth plays a key role in nutrient transfer from mother to baby. Mothers who are short in stature are known to have lower rates of amino acid synthesis in pregnancy. In these circumstances the ability of the placenta to transfer amino acids to the fetus may be critical.
Inflammatory response to routine vaccination at 6 and 13 weeks of age in relation to growth and body composition in low birthweight lambs

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Introduction: Serum amyloid A (SAA) is an acute phase reactant released during inflammation/infection, which is increased in obesity and associated with insulin resistance. Low birthweight is a risk factor for metabolic syndrome, therefore the inflammatory response to routine vaccination and its relationship with body composition during early life were examined in prenatally growth-restricted compared with normal birthweight lambs.

Materials/Methods: Singleton-bearing adolescent dams were offered control (C, n=20) or high (2.25 x C, n=25) nutrient intakes throughout pregnancy to induce normal or compromised prenatal growth trajectories, respectively. The resulting normal (N) or prenatally growth-restricted (PGR) lambs suckled their \textit{ad libitum} fed mothers until weaning at 11 weeks of age. At 6 and 13 weeks lambs received Heptavac P Plus® Clostridium/Pasteurella vaccine. Blood was sampled at 24 and 48 hours post-vaccination and SAA levels quantified by ELISA. Lamb growth rates were measured and body composition determined by dual-energy X-ray absorptiometry (DEXA) at weaning.

Results/Discussion: PGR lambs were 41% lighter than N at birth (3197±166.2 vs. 5425±166.1g, \(p<0.001\)). Fractional growth rate to weaning was elevated in PGR versus N groups (9.9±0.51 vs. 6.8±0.23\%, \(p<0.001\)) but absolute catch-up growth was not seen (liveweight at DEXA: 29±0.7 vs. 36±0.6kg, \(p<0.001\)). Nevertheless, percentage body fat was increased in PGR versus N (24.1±0.95 vs. 18.9±0.88\%, \(p<0.001\)) and in females versus males (24.3±0.90 vs. 18.6±0.84\%, \(p<0.001\)). At 6 weeks, both mean and peak SAA levels were increased in PGR versus N (peak: 357±42.4 vs. 219±49.0mg/l, \(p=0.005\)) and males versus females (356±53.3 vs. 233±41.1mg/l, \(p=0.014\)). At 13 weeks, SAA was independent of gender and PGR<N (69±13.4 vs. 141±34.9mg/l, \(p=0.051\)) and was weakly positively correlated with birthweight but weakly negatively correlated with percentage body fat (\(p<0.05\)).

Conclusion: Whilst inflammatory response to routine vaccination varied with prenatal growth status and postnatal age, no strong association with degree of adiposity was seen.
Mate drinking and the risk of small for gestational age at term. A case-control study in a Latinoamerican population

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Objectives: The objective of this study was to examine the association mate drinking habit (a typical drink from South America that includes caffeine) and different maternal characteristics as potential risk factor for small for gestational age in term pregnancies.

Materials: A hospital- based case control study was conducted between September 2009 and June 2010. A total of 110 cases and 220 controls were recruited. Cases consisted of small for gestational age defined as a term newborns with low birth weight that were hospitalized in our maternity during the study period. Controls were women who had a live term adequate for gestational age newborn randomly chosen from the same birth date of the case. Structured interviews of mothers of cases and controls during the first 24 hours after birth were conducted by specially trained Obstetrics and Gynecology residents.

Methods: A data base in ACCESS was specifically designed for the study and the data was analyzed in the software STATA. Univariate and bivariate analyses were performed for the most important variables. Students T-test was used for continuous variables and chi- square for categorical variables. Multivariate analyses for the most important variables were performed.

Results and Discussion: The mean maternal age in both groups was 25.1 years and 82% of the study population was Caucasian. We did not find any clinical and statistical difference in variables such as parity, educational level, and socio-economical status. About 82% of the population consumed mate during pregnancy and there was found an association with main outcome. We found in the 88.18 % of women who consumed mateína during de pregnancy, the association with small for gestational age at term versus 79.09 % in women who did not consumed (p= 0.042).

Conclusion: Mate drinking consumption during pregnancy is associated to small of gestational age at term.
Body weight and auditory startle response in rats permanently changed after reprogramming of diet and early exposure to MeHg.

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In this rat-study we hypothesized that maternal diet manipulation determines body weight and (neuro)development in the offspring and, with that, the vulnerability for environmental exposure to toxic substances, mimicked by chemical exposure to methyl mercury (MeHg). Three groups of female Wistar rats (n=24) were kept on a semi-synthetic control (i.e. ‘cont’) diet (n=24), a high-caloric (i.e. ‘high’) diet (15% butter oil, n=48) or a caloric restricted (deficient) (i.e. ‘def’) diet (75% of control diet, n=38) during a 6 weeks premating period and during mating (1 week) and gestation (3 weeks). To discriminate between dietary induced effects during gestation and lactation, pups were cross-fostered immediately after birth to dams of either the same or other two dietary groups to obtain 5 different groups, i.e. (gestation/LACTATION): cont/CONT; high/HIGH; def/DEF; def/HIGH and high/DEF; the def/HIGH group represents a rat model for the Dutch Famine. To study the effect of the diets on the vulnerability to early exposure to toxic substances, a selection of male (n=100) and female (n=100) pups was subcutaneously treated with saline (control) or MeHg (3 mg/kg body weight) from postnatal day (PN) 2-21. In time, physical and sensory landmarks, the auditory startle response (ASR) test and macroscopic brain examination were performed. Results showed that body weights of the F1-animals differed significantly between groups. Effects on body weights were slightly exacerbated by the exposure to MeHg and this effect maintained during adulthood. Dietary effects on development of landmarks, ASR and macroscopic brain examination were observed. Effects of MeHg on ASR were seen in especially females fed the reprogrammed def/HIGH diet (i.e. Dutch Famine rat-model). Moreover, sensory landmarks showed tendency towards delay in development after MeHg exposure.

It was concluded that maternal diet manipulation can have (transitory) effects which increase the vulnerability of the developing nervous system of the offspring to (subtle) environmental exposure, like MeHg.

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