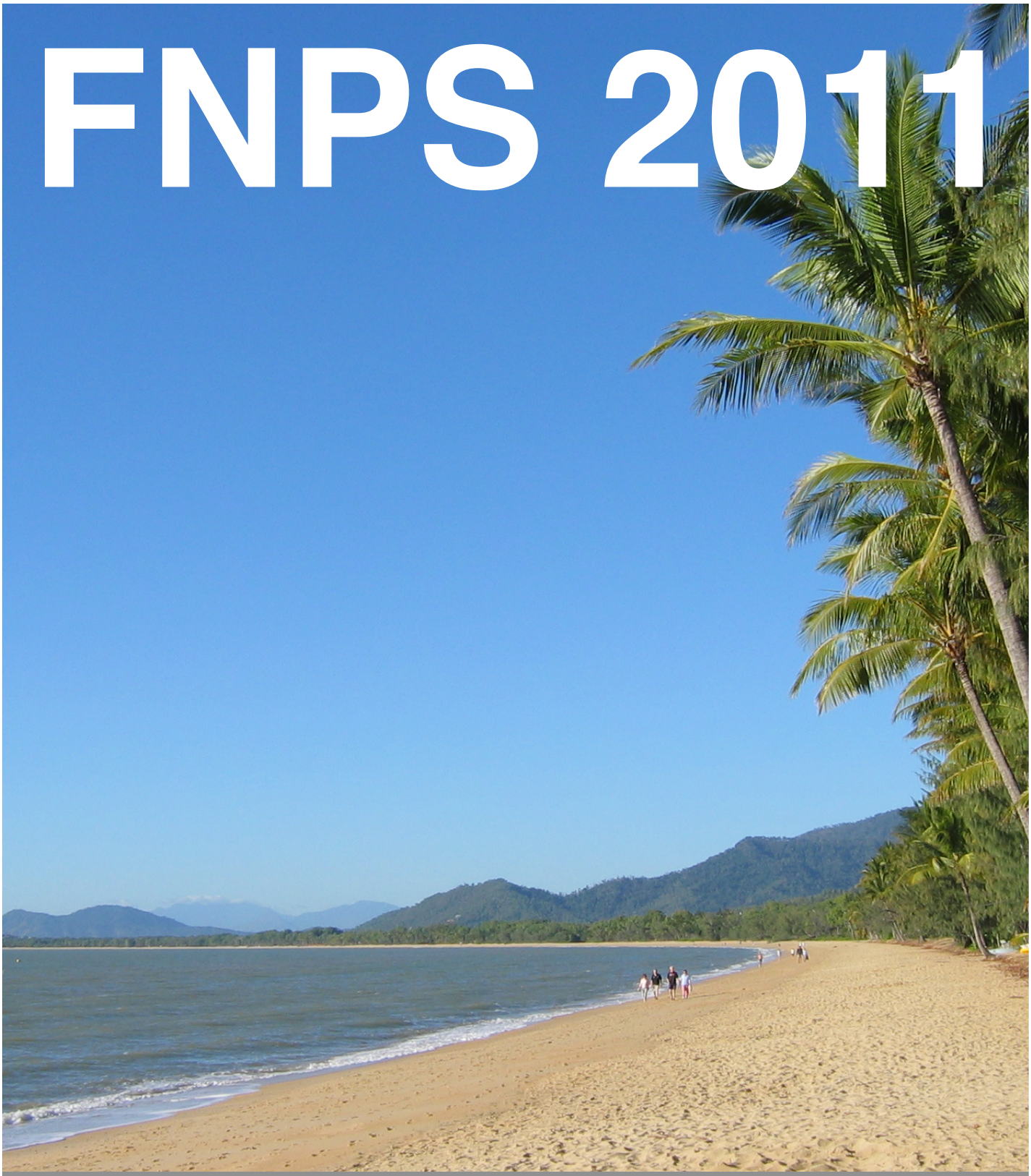
FNPS 2011



Proceedings of The 38th Meeting of The Fetal and Neonatal Physiological Society

10-13 July 2011

Grand Mercure Rockford Resort, Palm Cove, Queensland, Australia

Index

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 took the initiative and were made honorary members of the society in 1995.

The name of the annual conference (and society) has changed several times, reflecting the widening scope of the society:

1974-80	Conference on Fetal Breathing
1981-83	International Conference on Fetal Breathing and other Movements
1984-95	Society for the Study of Fetal Physiology
1996-present	Fetal and Neonatal Physiological Society

Over the years the society has maintained its informal character and a lack of rigid structures. Those who have attended at least one of the previous three meetings are members of the society and will be informed about the next meeting. Abstracts for the Annual Meeting are requested two months before the meeting and are compiled in the Book of Abstracts to encourage recent and preliminary data to be presented.

The Organizational Coordinator will be selected by the Organizational Committee and shall serve the three years. The Organizational Committee shall consist of representatives 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) Organizing Committee shall have the right to solicit funds in the name of the Society from organizations for the purpose of providing financial support for students and fellows-in-training to attend the meeting of the society.

Previous Meetings of the FNPS

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

Minutes of the FNPS Annual General Meeting

6 July 2010, Beaulieu, Hampshire, UK

The meeting was chaired by Prof Bill Parer, in the absence of Prof Laura Bennet (who was acting President, in the absence of Jan Nijhuis)

1. Acceptance of the minutes of the 2009 Annual General Meeting
The minutes of the Lake Arrowhead meeting were accepted with no amendments

2. Expression of gratitude to Lucy Green and the local organizing committee of the 2010 meeting
Prof Parer gave thanks on behalf of the Society to Dr Lucy Green and her organizing committee for making the 37th Annual meeting of the Society such a successful event.

3. Membership of FNPS Board
Emanuela Marinoni (Italy) and Karl Marsal (Sweden) are stepping down as members of the FNPS board. Appointment of suitable replacement members will occur in the near future. Jan Nihius remains as Chair of the FNPS board and Dino Giussani remains as scribe.

4. Publication of FNPS meeting abstracts
The FNPS board reached unanimous agreement that FNPS abstracts should not be formally published, so as not to threaten subsequent publication of the work. This decision was supported by members present.

5. FNPS archives
The board aims to have a complete set of the FNPS abstract books archived in the Northern hemisphere, at Cambridge University, and in the Southern hemisphere, at The Ritchie Centre at Monash University. Bill Parer is working on sourcing copies of the abstract books.

6. Future meetings:
   2011, July 10-13, Palm Cove, Queensland, Australia
   2012, The Netherlands
   2013, Chile

7. Student prizes
The following student prizes were announced during the closing of the meeting:
   Best oral presentation: Stopping the spread: connexion hemichannels in ischemic brain injury. Joanne Davidson, The University of Auckland, New Zealand.
   Best poster presentation: Maternal protein or lipid undernutrition impact on development of the mouse blastocyst. Francesca Lock, University of Southampton, United Kingdom.

8. Other business
None

Tim Moss
Acting FNPS Scribe

39TH ANNUAL MEETING OF THE Fetal and Neonatal Physiological SOCIETY

8-11 JULY 2012 - UTRECHT, THE NETHERLANDS

ORGANISING COMMITTEE

Jan Derks, Joepe Kaandorp, Edu Mulder, Deodata Tijsseling, Gerard Visser

Welcome

A warm welcome to Australia and to Palm Cove for the 38th Meeting of the Fetal and Neonatal Physiological Society. Our aim for this meeting is to provide an environment for exciting discussions to further examine how clinicians and scientists alike can work together towards a healthy pregnancy and the best start for babies.

This year we bring together representatives from 11 countries covering 6 continents. Reading through the abstracts confirms that 2010 and 2011 have been very busy for all of us. Our meeting contains a full schedule of oral communications and posters that will provide plenty of stimulating discussions both in the conference hall and around the poolside. Our thoughts over the past 12 months have also been with our colleagues and friends in Japan and Christchurch in New Zealand – the FNPS community is thinking of you and your families as you rebuild your homes and lives.

In addition to the official communications sessions, as is traditional for FNPS meetings, the conference at Palm Cove will provide and encourage a variety of opportunities for socialising. As always, the FNPS sporting event remains top-secret but nothing to fear; our student representatives have been organising the sports and we encourage participation by everyone (tip - bring your beach gear).

Finally, some words of thanks. The FNPS 2011 has been well supported by a variety of organisations. In particular, we are very grateful to The Ritchie Centre, Monash University and the Faculty of Medicine, Nursing and Health Sciences who are proud gold sponsors of FNPS 2011 and The Dawes Lecture. A very big thank-you to Graham Jenkin, Kelly Crossley and David Walker for their assistance in raising sponsorship and thanks to Tim Moss for getting our first ever 'app' up and running. Thank-you also to Maree Overall and the ASN Events team for fabulous support in all aspects of ensuring our meeting has run a smooth course to Palm Cove.

Thank you for supporting FNPS 2011 and we hope you have an enjoyable conference.

Jon Hirst and Suzie Miller
On behalf of the FNPS 2011 Local Organising Committee

Organising Committee

Local Organising Committee 2011
Laura Bennet
Richard Harding
Graham Jenkin
Barbara Lingwood
Tim Moss
Beverley Mulhausler
Jane Pillow
David Walker
Robert Galinsky (student representative)
Amy Sutherland (student representative)
Udani Ratnayake (student representative)

FNPS Board Members 2011
Jan Nihius (Chair, The Netherlands)
Dino Giussani (Scribe, UK)
Anibal Llanos (Chile)
Brian Koos (USA)
Carina Mallard (Sweden)
Donald Peebles (UK)
Dan Rurak (Canada)
Lucy Green (UK)
Laura Bennet (New Zealand)
Luc Zimmermann (The Netherlands)
Bill (J.T.) Parer (USA)
Tomoaki Ikeda (Japan)
Tim Moss (Australia)
Charles Wood (USA)

Sponsors

Gold Sponsors

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Medicine, Nursing and Health Sciences

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the RITCHIE CENTRE

Silver Sponsors

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Cell Care

MBc max biocare

Bronze Sponsor

MACS Miltenyi Biotec

Delegate Information

THE REGISTRATION DESK

The Registration desk will be located in the Foyer. Any enquiries about registration or the conference can be directed to volunteer staff there. The registration desk hours are:

Sunday:	2:00pm – 6:00pm
Monday:	8:00am – 4:00pm
Tuesday:	8:00am – 5:30pm
Wednesday:	8:30am – 1:00pm

Note that the conference concludes at 1:00pm on Wednesday.

WHAT YOUR REGISTRATION INCLUDES:

The Delegate registrations include:

- Access to the sessions of your choice;
- Conference Dinner
- Welcome Poolside Party
- Aussie BBQ Dinner
- Conference program book and satchel;
- Welcome Reception Monday evening;
- Meal breaks

VENUE

- All scientific sessions will take place in the Hibiscus Room
- All morning teas, lunches, afternoon teas, trade and poster sessions will be in the Orchid Room

ACCOMMODATION

Accommodation has been offered at the Grand Mercure Rockford Esplanade and the Novotel Palm Cove; the reception desk will be able to assist you with any enquiry about accommodation. A shuttle bus is provided to convey delegates between the Grand Mercure and the Novotel. Check in time is from 2:00pm on the day of arrival. Individuals will be required to settle their room accounts with the hotel on the morning of checkout. Check out time is 10:00am. Don't forget that those people who opted to only pay for a portion of their accommodation in advance can expect to have to settle the balance with the hotel, along with any other incidental expenses incurred upon checkout.

SOCIAL PROGRAM:

Welcome Reception – Sunday 10th July, 7:00pm – 9:00pm, Poolside
Aussie BBQ Dinner – Monday 11th July, 6:30pm – 10:00pm
Conference Dinner – Tuesday 12th July, 7:00pm – 11:30pm, at the conference venue.

SPEAKER PREPARATION INSTRUCTIONS

The audio-visual equipment is being supplied and manned by our own FNPS delegates. It is the conference preference to have **ALL** talks pre-loaded to either the common PC or your own Mac. As per instructions already supplied, you should give your talk on a usb drive to the technicians well before the session you are participating in so it can be loaded and tested. A technician will always be attending the laptop during sessions so it is always possible to find them in the plenary room immediately prior and after any session.

DISPLAYING YOUR POSTER

Posters will be displayed for the session as allocated, amongst the exhibition area, in the Orchid Room. Please locate your abstract number for correct positioning. The maximum size provided is 1m wide by 1.2m high. The approved way of attaching your poster is with Velcro which will already be at your poster location. Additional Velcro is available from the registration desk if required.

Storing a baby's cord blood stem cells is the one thing parents don't want to forget.

Worldwide, more and more people are choosing to store their baby's cord blood stem cells for use in the treatment of a range of diseases, including those of the blood and immune system, and some cancers. As medical research continues to unlock further potential uses for stem cells, it's one of the most important decisions parents can make for their baby. They only have one opportunity to store them – at the time of delivery. To learn more about the benefits for your patients or to become a cord blood collector call **1800 071 075** or visit **cellcareaustralia.com**

Cell Care

DAWES Lecture

GEOFFREY DAWES LECTURE 2011

This lecture series is given in memory of the late Geoffrey Dawes and is designed to stimulate, educate and entertain on a topic in reproduction or perinatology, but as was Geoffrey's wont, can be quite 'left field' in nature.

"From Oxford to Melbourne, two extraordinary contributions."

Richard Harding discussing David Walker…
And David Walker discussing Richard Harding.

Richard Harding
Senior Principal Research Fellow,
Professorial Fellow
Department of Anatomy and Developmental Biology
Monash University

David Walker
Senior Research Fellow
The Ritchie Centre
Monash Institute of Medical Research
Monash University

Proud sponsors of the Dawes Lecture 2011.

ABSTRACT AWARDS
Tania Gunn Memorial Prize

To be awarded to the best oral presentation by a student ($500NZ) and the best oral presentation by an early postdoctoral fellow ($500NZ).

This award is made in memory of the late Professor Tania Gunn (1932-99). Tania graduated the Otago School of Medicine in 1955, and after a period out of medicine, retrained in neonatology in the 1970's at Montreal's Children's Hospital. She was appointed in 1978 as Auckland's first specialist neonatologist. From 1981 she undertook ground-breaking studies of the control of thermo-regulation at birth and then on the resuscitation of newborns and infants. She is particularly remembered for her central role in the first ever randomized safety study of therapeutic hypothermia for babies with acute encephalopathy. Her enthusiasm for science and her commitment to the health of newborn babies is rarely matched and will be sorely missed.

Dining and Activities around the Venue

Mii Spa
Located at the Sea Temple resort The Mii Spa Offers three beautifully appointed treatment rooms, two with private outside verandas leading onto a man-made rainforest, where you can relax and unwind after your treatment. Ergonomically designed massage tables will ensure the neck, spine and leg muscles are cradled in complete comfort while taking the strain off any pressure points as well as heated to ensure the body's temperature remains constant throughout the entire treatment.

Contact Details
Web: http://www.mirvachotels.com/sea-temple-resort-palm-cove/mii-spa
Email: miispa@strspc.mirvac.com.au
Phone: + 61 7 4059 9613.

Sky Rail:
Skyrail Rainforest Cableway located just a few minutes' drive from Palm Cove is truly one of the most magnificent experiences for any holiday in far north Queensland The Skyrail experience, spanning 7.5kms over pristine rainforest, allows you to explore the wonders of an ancient tropical rainforests and learn about one of the most botanically fascinating and diverse areas on earth. Gliding just metres above the rainforest canopy in comfortable 6-person gondola cabins the Skyrail journey immerses you in an intimate rainforest experience where you'll see, hear, smell and become part of the tropical rainforest environment.

Contact Details:
Web: http://www.skyrail.com.au/
Email: mail@skyrail.com.au
Phone: 61 7 4038 1555

Hot Air Balloons:
Not many people know but the hot air ballooning capital of the world, is Cairns/Palm Cove. Up to 12 balloons a morning, go floating over the Atherton Tablelands. Even better, balloon rides here are up to 48% cheaper than Syd/Melb/Canberra at only $175pp including flight, return transfers, full hot brekkie & champagne toasts. Seeing all the balloons inflating is an AMAZING way to start the day. Mention STAYZ & get a FREE Boxed Set of Champagne Flutes.

Contact Details
Web: http://www.hotair.com.au
Email: cairns@hotair.com.au
Phone: + 61 7 4039 9900

Barron River Gorge:
The Barron River Gorge is located 10 minutes' drive from Palm Cove and lies along a traditional pathway used by the original indigenous Djabugay people. The spectacular Barron Falls, further upstream, are considered a sacred site. The famous Kuranda Railway and Skyrail Cableway also traverse sections of the gorge. Stoney Creek on the southern side of the Gorge is home to one of North Queensland's most beautiful national parks, complete with isolated waterfalls and swimming holes in a tropical wonderland.

Contact details
Web: http://www.wanggulay.com/
Phone: +61 7 4039 1461

Local Restaurants

Casmar Restaurant & Bar
If you are in the Palm Cove/Port Douglas/Cairns area and looking for seafood, then the Casmar cannot be surpassed. With a Seafood Platter for 2 at $90 this is one of the cheapest in the area and certainly worth every cent. With prawns, Barramundi, bugs, calamari and oysters, it is spectacular.
Address: Cr Harpa St & Williams Esplanade Palm Cove
Ph: (07) 4059 0013
Web: www.casmar.com.au/

Nu-Nu Restaurant
Recline on oversized banquettes and watch the sheer silver and ice blue curtains waft in the breeze against a backdrop of glistening sea views. If that isn't enough to put you in a peaceful frame of mind, the professional and polished service and delicious taste sensations certainly will be. For a unique and delicious breakfast, try the 'toastie' of poached rhubarb and apple on sweet challah bread with vanilla cream. Lunch and dinner options are no less appealing. Among them, blue swimmer crab and avocado sandwiches, roasted Spring Bay scallops or pork and bean-shoot egg-nets with pink pomelo (grapefruit) and a chilli coconut caramel sauce.
Address: 123 Williams Esplanade, Palm Cove QLD 4879
Phone: (07) 4059 1880
Web: www.nunu.com.au/

Apres Beach Bar and Grill
The friendly and inviting atmosphere of Apres Beach Bar & Grill has earned us the reputation of being one of Cairns and North Queensland's premier meeting places, for both locals and visitors alike. Overlooking beautiful Palm Cove beach, only 20 minutes north of Cairns, you can meet for a meal, or mingle over a drink and soak up the buzz that defines the Apres style.
Address: 119 Williams Esplanade Palm Cove Queensland 4879 Australia
Ph: 07 4059 2000
Web: www.apresbeachbar.com.au/

Sponsor and Exhibitor Listing

Monash University **Gold Sponsor**
Website**: www.monash.edu.au/**
Monash is an energetic and dynamic university committed to quality education, outstanding research and international engagement. Our researchers are committed to finding solutions for 21st Century challenges such as climate change, water shortage, cancer, diabetes, obesity and those posed by terrorism. We offer our students access to a rich and international learning experience. With a presence in Australia, Asia, South Africa and Europe, Monash fosters a truly global perspective. At Monash University, education is about how ideas change people and how people change the world**.**

Office of the DVC Research, Monash University
Website**: www.monash.edu.au/international/dvc/stafflist.html**
The Fetal and Neonatal Physiological Society gratefully acknowledges the support of the Office of the Deputy Vice Chancellor (Research), Monash University, Australia, for its contribution to the travel costs of Richard Harding and David Walker of Monash University to enable them to jointly deliver the Dawes Lecture 2011.

Faculty of Medicine, Nursing and Health Sciences, Monash University **Gold Sponsor**
Website: **www.med.monash.edu.au/**
The Faculty of Medicine, Nursing and Health Sciences at Monash University is recognised as a leader in biomedical research, and is one of the highest ranked institutions for teaching and learning in its field in Australia. Both the Times Higher Education and QS rankings place Monash in the world's top 50 universities for the life science disciplines. A member of the 'Group of Eight' of Australia's research intensive universities, Monash is prominent in the global M8 Alliance of academic health centres and medical universities, and is honoured to hold the Co-Presidency of the 2011 World Health Summit in Berlin**.**

The Ritchie Centre, Monash Institute of Medical Reaearch **Gold Sponsor**
Website: **www.monashinstitute.org/centres/rcbh/baby-health.html**
The Ritchie Centre is proud to be a major sponsor of the 2011 Fetal and Neonatal Physiological Society meeting. The Ritchie Centre, located within the Monash Institute of Medical Research and the Department of Obstetrics and Gynaecology of Monash University, is Australia's leading Fetal and Neonatal Research Centre. The Ritchie Centre combines basic science research and clinical medicine to improve the health and wellbeing of women and babies. The close collaboration between scientists of The Ritchie Centre, The Melbourne Children's Sleep Unit and clinicians at the Monash Newborn Unit at Monash Medical Centre offers a unique setting where research developments can be rapidly applied for the benefit of seriously ill babies and children.

ADInstruments **Exhibitor**

Website: **www.adinstruments.com**

Established in 1988, ADInstruments is a world-leading provider of computer-based data acquisition and analysis systems for Life Science. Our products enable users to record and analyse life science data quickly and efficiently. The PowerLab system is used in universities, hospitals, research institutes, pharmaceutical companies, contract research organizations and other private industry research sectors.

Cell Care Australia **Silver Sponsor**

Website: **www.cellcareaustralia.com**

Cord blood is the blood that remains in a newborn's umbilical cord and placenta after the umbilical cord has been cut. What used to be medical waste is now understood to be rich in stem and other cells that may be of therapeutic benefit for the child some time later in his or her life.

Cell Care is Australia's largest and premier private cord blood banking service. Providing excellence in all aspects of cord blood banking, Australian families can be secure in the knowledge that they have provided their children with the best possible opportunity to benefit from future medical breakthroughs in stem cell research.

Fisher & Paykel Health Care **Silver Sponsor**

Website: **www.fphcare.com**

Fisher & Paykel Healthcare has a range of products including specialised circuits, humidifiers, CosyCots, infant warmers, Neopuff T-piece resuscitators, resuscitation masks and Bubble CPAP. These products are designed to provide infant care whether it is delivering optimal levels of humidity, providing safe and effective resuscitation, or meeting energy equipment's.

Max Biocare **Silver Sponsor | Exhibitor**

Website: **www.maxbiocare.com**

Max Biocare Pty Ltd. is an independent Australian owned and operated company committed to providing health solutions in the form of scientifically validated natural medicines. The company's comprehensive product range is manufactured in accordance with the Australian TGA's GMP standards. A key area of the company's focus is providing a healthy start to life. This includes products for women's reproductive health, pregnancy and lactation, as well as products for newborns and young growing children. Max Biocare is committed to providing consumers with quality and innovative natural medicines, and also takes a special interest in supporting scientific research.

Miltenyi Biotec **Bronze Sponsor**

Website: **www.miltenyibiotec.com**

Miltenyi Biotec is the global market leader in magnetic cell separation. MACS® Technology is the gold standard in cell used by researchers and clinicians all over the world. Superparamagnetic MACS MicroBeads and convenient MACS Columns facilitate targeted research on isolated cell types or subsets – in any lab. Besides cell isolation, this technology enables highly specific molecule purifications and revolutionary on-column enzymatic reactions.

We are developing cellular therapeutics to meet unmet medical needs and are committed to delivering the promise of cellular medicine in the fields of organ regeneration, immune modulation and transplantation.

Program

Sunday, 10 July 2011

Registration
2:00 PM - 6:00 PM Foyer

Emerging Research/Emerging Researchers
4:00 PM - 5:30 PM Hibiscus Room
Chairs: Laura Bennet & Jan Nijhuis

Session Sponsored by MONASH INSTITUTE OF MEDICAL RESEARCH the RITCHIE CENTRE

4:00pm **Erin McGillick**
Role of Glucose in Surfactant Protein mRNA Expression in the Fetal Lung *abs#001*

4:15pm **Deodata Tijsseling**
Antenatal glucocorticoid treatment reduces neuronal density in the human hippocampus *abs#002*

4:30pm **Tamara Yawno**
The Effects of Human Amnion Epithelial Cells (hAECs) on Fetal Brain Injury Induced by Lipopolysaccharide *abs#003*

4:45pm **Robert Galinsky**
Effect of intrauterine inflammation on fetal and neonatal cardiopulmonary and cerebral circulation *abs#004*

5:00pm **Paul Drury**
Dopamine is ineffective for cardiovascular support after asphyxia in near-term fetal sheep *abs#005*

5:15pm **Andrew Kane**
Xanthine Oxidase-Derived Reactive Oxygen Species Affect Cardiovascular Function in the Fetus *abs#006*

Dawes Lecture
5:30 PM - 7:00 PM Hibiscus Room

Session Sponsored by MONASH University Medicine, Nursing and Health Sciences
and Office of the DVC Research, Monash University

Welcome Reception
7:00 PM - 9:00 PM Poolside

Monday, 11 July 2011

Registration
8:00 AM - 4:00 PM Foyer

Brain and Behaviour
8:45 AM - 10:30 PM Hibiscus Room
Chairs: Alistair Gunn & Emily Camm

8:45am **Ryuta Kitanishi**
Diffuse white matter injury of premature brain in the chronically instrumented fetal sheep *abs#007*

9:00am **Laura Thei**
Systemic Inhibition of Extracellular Signal-Regulated Kinase (ERK) has a Protective Effect in Neonatal Cerebral White Matter Injury *abs#008*

9:15am **Amy Sutherland**
Melatonin as a neuroprotectant in the growth restricted ovine fetus following glucocorticoid administration *abs#009*

9:30am **Margie Castillo-Melendez**
Maternal diet supplementation with creatine from mid-gestation protects the newborn spiny mouse brain from birth hypoxia *abs#010*

9:45am **Mary Tolcos**
Cerebral myelination in IUGR: Not just myelin basic protein *abs#011*

10:00am **Joanne Davidson**
Sleep architecture of preterm fetal sheep and the effect of antenatal glucocorticoids *abs#012*

10:15am **Alistair Gunn**
Maturation of mitochondrial redox response to profound intrauterine hypoxia *abs#013*

Tea Break
10:30 AM - 11:00 AM Orchid Room

Poster Session 1 Orals
11:00 AM - 12:00 PM Hibiscus Room
Chairs: Donald Pebbles & Hannah Palliser

Euan Wallace
Activin: towards a new mouse model of preeclampsia *abs#101*

Foula Sozo
Does daily ethanol exposure during late ovine gestation alter fetal liver development? *abs#102*

Min Kim
Sex-specific structural maturation of the myocardium in preterm piglets *abs#103*

Carlie Cullen
Long Term Behavioural Outcomes of Chronic Low Dose Prenatal Ethanol Exposure *abs#104*

Takushi Hanita
Does endotoxin exposure during late gestation cause brain injury? *abs#105*

Hayley Dickinson
Histological, phenotypic and proliferative potential of hematopoietic cell populations in the fetal, neonatal and adult spiny mouse *abs#106*

Anzari Atik
Does high dose chronic caffeine treatment affect physiological status and brain development of the immature fetus? *abs#107*

Luke Weaver-Mikaere
Chronic Exposure to TNF-α in an *in vitro* Ovine Model of Preterm Brain Injury: Effects on Glutamate Receptors *abs#108*

Mary Tolcos
Increased cell proliferation and angiogenesis in the fetal subventricular zone is correlated with

the severity of growth restriction *abs#109*

Vivian Nguyen

Effect of chronic low dose alcohol exposure *in utero* in the hearts of rat offspring *abs#110*

Eric Tremblay

Differential Effects of Epidermal Growth Factor on the Inflammatory Response along the Developing Human Intestine *abs#111*

Reetu Singh

Urinary concentrating defect in response to exogenous arginine vasopressin infusion and water deprivation in male sheep following fetal uninephrectomy *abs#112*

Kristy Weir

Changes in fetal skin morphology, permeability and *Tgm1* gene expression following maternal glucocorticoid exposure in the mouse *abs#113*

Dawn Elder

Do convalescent preterm infants exhibit heart rate variability? *abs#114*

Noreen Ishak

Is the male disadvantage in respiratory outcome following preterm birth a result of altered surfactant composition? *abs#115*

Stephen Anderson

Osteochondritis Dissecans (OCD) in Australian Thoroughbred horses is associated with chronic hypoinsulaemia in early post-natal life *abs#116*

Lindsea Booth

Altered cardiovascular and neural responses of the preterm fetus to asphyxia after chronic exposure to LPS *abs#117*

Joepe Kaandorp

Antenatal allopurinol protects the fetal heart and brain after acute birth asphyxia in late gestation fetal sheep *abs#118*

Stacey Ellery

Does Birth Asphyxia in the Spiny Mouse Lead to Neonatal Renal Failure? *abs#119*

Julie Owens

Maternal Influences on Placental Epigenetic Signatures in Pregnancies of Overweight and Obese Women *abs#120*

Poster Session 1 Viewing
12:00 PM - 1:00 PM Orchid Room

Lunch
1:00 PM - 2:00 PM

Placenta
2:00 PM - 3:45 PM Hibiscus Room
Chairs: Dino Giussani & Hayley Dickinson

Session Sponsored by Cell Care

2:00pm **Hannah Palliser**
Vulnerability of IUGR pregnancies to preterm labour: Activation of inflammatory prostaglandin pathway *abs#014*

2:15pm **Yuichiro Miura**
Development of an artificial placenta : Pumpless arteriovenous extracorporeal life support in a premature lamb model *abs#015*

2:30pm **Eugenie Lumbers**
Roles of the placental renin-angiotensin system in placental vasculogenesis? *abs#016*

2:45pm **Rebecca Lim**
Role of activin A in the pathophysiology of preeclampsia *abs#017*

3:00pm **Emilio Herrera**
Vasodilator effect of hydrogen sulphide (H_2S) in the perfused human placenta *abs#018*

3:15pm **Tamas Zakar**
Epigenetic regulation of inflammatory genes in the amnion during pregnancy *abs#019*

3:30pm **Michael Stark**
Eicosapentanoic acid (EPA) is more effective than docosahexanoic acid (DHA) in inhibiting LPS-induced lipid hydroperoxide production and oxidative DNA damage in the placenta *abs#020*

Special Activity
4:30 PM - 6:30 PM Surprise!

Aussie BBQ Dinner
6:30 PM - 10:00 PM Poolside

Tuesday, 12 July 2011

Registration
8:30 AM - 5:30 PM Foyer

Lungs
8:45 AM - 10:30 PM Hibiscus Room
Chairs: Tim Moss & Foula Sozo

Session Sponsored by Fisher&Paykel HEALTHCARE

8:45am **Alana Westover**
Elucidating the role of prostaglandins in the fetal inflammatory response *abs#021*

9:00am **Patricia Vosdoganes**
Human Amnion Epithelial Cells modulate the pulmonary structural and inflammatory responses to intrauterine inflammation in fetal sheep *abs#022*

9:15am **Sheena Bouch**
Lung Development in Postnatal Rats Following Maternal Vitamin D Deficiency *abs#023*

9:30am **Megan O'Reilly**
Inhalation of hyperoxic gas in the neonatal period: long-term pulmonary implications *abs#024*

9:45am **Nadine Brew**
The potential role of repair genes Urokinase Receptor and Metallothionein in the repair of lung structure following ventilation induced injury in the immature lung *abs#025*

10:00am **K Crossley**
The effect of acute caffeine administration on pulmonary function in ventilated very preterm lambs *abs#026*

10:15am **Robert De Matteo**
Persistent alterations in the lungs of adolescent preterm lambs following intrauterine endotoxin exposure *abs#027*

Tea Break
10:30 AM - 11:00 AM Orchid Room

Poster Session 2 Viewing
11:00 AM - 12:30 PM Orchid Room

James Aridas
Correlating histological damage with neuroradiological imaging in a term lamb model of birth asphyxia *abs#121*

Mary Black
Cardiomyocyte growth and maturation during mid to late gestation and the effect of preterm birth *abs#122*

Nadine Brew
Lung Repair after Ventilator-Induced Injury of the Extremely Preterm Ovine Lung *abs#123*

David Cannata
Lactoferrin: A necessary inclusion for pregnancy and newborn supplements *abs#124*

Kelly Crossley
The effect of mechanical ventilation and anaesthesia on the preterm ovine brain *abs#125*

Paul Drury
Chronic in utero exposure to low-dose endotoxin impairs EEG maturation *abs#126*

Rebecca Dyson
H_2S; A novel player in the transitional microcirculation of preterm neonates? *abs#127*

Robert Galinsky
Markers of lung injury in mechanically ventilated preterm sheep exposed to intrauterine inflammation *abs#128*

Oksan Gezmish
Vitamin D deficiency, commencing in utero, affects cardiac function and increases the

susceptibility to myocardial ischemia/reperfusion injury in adulthood *abs#129*

Emilio Herrera

Rho-kinase inhibition modifies pulmonary vascular reactivity in chronically hypoxic neonatal lambs in the Andean *altiplano* *abs#130*

Yoshiyasu Hombo

A computer simulation showed that amniotic fluid pressure in the third trimester would be kept stable agaist uterine tonus by automatic change of its volume *abs#131*

Rosemary Horne

Effects of preterm birth on the maturation of autonomic control during sleep in infancy *abs#132*

Meredith Kelleher

Neurosteroid replacement therapy in the preterm neonate *abs#133*

Eugenia Koulaeva

Follistatin and the treatment of hyperoxia-induced lung injury in neonatal mice *abs#134*

Domenic LaRosa

Could the newborn heart be resistant to hypoxic injury? *abs#135*

Rebecca Lim

Oxidative stress induced unfolded protein response in preeclampsia *abs#136*

Rebecca Lim

Role of regulatory T cells s in human amnion epithelial cell mediated lung repair *abs#137*

Rebecca Lim

Role of macrophages in human amnion epithelial cell mediated lung repair *abs#138*

Eugenie Lumbers

Effects of fetal sex on prorenin production by the decidua *abs#139*

Monique Mortale

Do Neurosteroids Protect the Developing Brain of the Growth Restricted Fetus? *abs#140*

Bree O'Connell

Pregnancy, placentation and fetal growth in the spiny mouse *abs#141*

Chan-Wook Park

Histologic chorioamnionitis is present in approximately one-fourth of patients with preterm labor and intact membranes and low AF WBC counts and is a significant risk factor for intra-amniotic inflammation and RDS of newborns *abs#142*

Julia Pitcher

Gestation length and fetal growth have different effects on corticospinal excitability and motor skill development in children *abs#143*

Michael Stark

Dose response effect of docosahexanoic acid supplementation on plasma markers of oxidative stress in preterm neonates (28-32 weeks) from initiation of enteral feeds to day 28 of life *abs#144*

Laura Thei

Signal Transduction and Activation of Transcription factor 3 (STAT3) mediates neonatal hypoxic ischaemic brain injury *abs#145*

Jochem Van Der Pol

Expression of Angiopoietin-1 & 2 in the mid-gestation fetal sheep brain following in utero global hypoxia *abs#146*

Kom Yin

Compensatory growth of healthy cardiomyocytes in diseased fetal hearts: effects on cardiomyocyte number at birth *abs#147*

Tamas Zakar

Progesterone withdrawal induces intrauterine PGHS-1 expression in the pregnant guinea pig *abs#148*

Lunch

12:30 PM - 1:30 PM Lunch area tbc

Cardiovascular

1:30 PM - 3:15 PM Hibiscus Room

Chairs: M Jane Black & Karen Gibson

1:30pm **Miriam Nyberg**
Superior vena cava blood flow in the human fetus and the effect of fetal breathing movements *abs#028*

1:45pm **M Black**
Effects of ibuprofen treatment on the developing preterm baboon kidney *abs#029*

2:00pm **D Giussani**
Heart Disease Link to Prenatal Hypoxia and Oxidative Stress *abs#030*

2:15pm **Laura Bennet**
The effect of melatonin on cardiac performance during asphyxia in the preterm fetal sheep *abs#031*

2:30pm **Janna Morrison**
Increased phosphorylated CaMKII may be responsible for cardiac hypertrophy in LBW lambs *abs#032*

2:45pm **Barbara Lingwood**
Coronary and aortic flow in response to changes in preload and afterload in the isolated preterm piglet heart *abs#033*

3:00pm **Graeme Polglase**
Acute inflammation *in utero* adversely alters cerebral blood flow (CBF) and brain injury in preterm lambs *abs#034*

Tea Break

3:15 PM - 3:45 PM Orchid Room

Neonatal Wellbeing

3:45 PM - 5:15 PM Hibiscus Room

Chairs: Alan Bocking & Michael Stark

3:45pm **Nicolette Hodyl**
Asthma during pregnancy increases the risk of significant neonatal morbidity *abs#035*

4:00pm **Julia Pitcher**
Children born preterm have reduced long term depression (LTD)-like neuroplasticity *abs#036*

4:15pm **Shinji Katsuragi**
Mechanism of reduction of umbilical arterial metabolic acidemia following application of a standardized rule-based FHR management schema *abs#037*

4:30pm **Rosemary Horne**
Why is sleeping in the prone position a risk for the Sudden Infant Death Syndrome? *abs#038*

4:45pm **Alison Kent**
Podocytes are found in the urine of preterm infants receiving indomethacin treatment suggesting drug induced glomerular injury *abs#039*

5:00pm **Meredith Kelleher**
Sex and progesterone: improving preterm survival *abs#040*

Conference Dinner

7:00 PM - 12:00 AM Meleluca Room

Wednesday, 13 July 2011

Registration 8:30 AM - 1:00 PM Foyer

Developmental Programming

8:45 AM - 10:30 AM Hibiscus Room

Chairs: Beverley Muhlausler & Tamas Zakar

8:45am **Megan Probyn**
Prenatal low dose ethanol exposure alters glucose homeostasis in rats *abs#041*

9:00am **James Brien**
Effects of voluntary exercise on ethanol neurobehavioural teratogenicity in the guinea pig *abs#042*

9:15am **Julie Owens**
Diet induced paternal obesity impairs the metabolic and reproductive health of two subsequent generations *abs#043*

9:30am **Youguo Niu**
Statins Prevent Adverse Effects of Postnatal Dexamethasone Therapy on Cardiovascular Function in Weanling Rats *abs#044*

9:45am **Emily Camm**
Developmental programming of neurological disease: Role of adverse pre- and neo-natal conditions and oxidative stress *abs#045*

10:00am **Shervi Lie**
Periconceptional Undernutrition Alters Insulin Signalling in Muscle from Late Gestation Sheep Fetus *abs#046*

10:15am **Ciara Lusby**
Prenatal Chronic Hypoxia Affects Insulin Signalling in Adult Male Rat Offspring *abs#047*

Tea Break

10:30 AM - 11:00 PM Orchid Room

Maternal Environment & Fetal Growth

11:00 AM - 12:45 PM Hibiscus Room

Chairs: Vicki Clifton & Kelly Crossley

11:00am **Udani Ratnayake**
Changes in the intrauterine environment due to maternal poly I:C administration in the spiny mouse *abs#048*

11:15am **Tuan-Ahn Nguyen**
Lack of Fluoxetine Effects in Postnatal Lambs *abs#049*

11:30am **Marianne Tare**
Prenatal alcohol exposure causes persistent changes in vascular function and passive mechanical arterial wall properties in the offspring *abs#050*

11:45am **Yourka Tchoukalova**
Maternal nutrient restriction alters expression of genes that regulate baboon fetal preadipocyte maturation *abs#051*

12:00pm **David Carr**
Prenatal Ad.VEGF gene therapy increases fetal growth velocity in the absence of a measurable effect on uterine blood flow in an ovine paradigm of fetal growth restriction *abs#052*

12:15pm **Gert Maritz**
Tomato juice protects the lungs of the offspring of female rats exposed to nicotine during gestation and lactation *abs#053*

12:30pm **Beverly Muhlhausler**
The effects of maternal high fat feeding on body fat mass and susceptibility to diet induced obesity can be reversed by interventions during the neonatal period *abs#054*

Meeting Close 12:45 PM - 1:00 PM Hibiscus Room

Abstracts

ORAL

001

ROLE OF GLUCOSE IN SURFACTANT PROTEIN MRNA EXPRESSION IN THE FETAL LUNG

E. V. McGillick, S. Orgeig, C. McMillen, J. L. Morrison

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Background: In the past decade, there has been a substantial increase in the proportion of women entering pregnancy as overweight or obese which predisposes to multiple obstetric complications including gestational diabetes and preterm birth. Obese women are less insulin sensitive than lean and overweight women and are therefore at an increased risk of gestational diabetes. Increased circulating fetal glucose and insulin concentrations are potential inhibitors of fetal lung maturation *in utero* and may contribute to the pathogenesis of respiratory distress syndrome observed in infants of diabetic mothers.

Hypothesis: High plasma glucose and insulin concentrations in the late gestation sheep fetus will result in decreased surfactant protein mRNA expression in the lung.

Methods: Vascular catheters were implanted into the ewe and fetus. At 130d gestation (term 150±3d), a glucose solution (50% dextrose 250g/L in saline) was infused continuously at an initial rate of 1.9ml/h for 24h, increased in a stepwise manner by 1.9ml/h per day for 3d with a final infusion rate of 7.5ml/h until post mortem at 140d gestation (n=8). Control fetuses received a saline infusion (n=7). The relative abundance of SP-A, -B, -C and -D mRNA transcripts in fetal lung samples was measured by qRT-PCR. Data were analyzed by a student's t-test and $P<0.05$ was considered statistically significant.

Results: The glucose infused fetuses had higher plasma glucose and insulin concentrations ($P<0.05$) than the saline infused fetuses throughout the infusion period. Lung SP-A and SP-C mRNA expression were reduced ($P<0.05$) in the glucose infused fetuses.

Conclusions: Increased fetal glucose and insulin concentrations result in a down regulation of surfactant protein mRNA expression in the lung of the late gestation sheep fetus which provides evidence for the link between abnormal glycemic control *in utero* and RDS observed in infants of obese mothers.

002

ANTENATAL GLUCOCORTICOID TREATMENT REDUCES NEURONAL DENSITY IN THE HUMAN HIPPOCAMPUS

D. Tijsseling[1], L. D.E. Wijnberger[2], J. B. Derks[1], C. T.J. Van Velthoven[3], W. B. De Vries[1], F. Van Bel[1], P. G.J. Nikkels[4], G. H.A. Visser[1]

[1]Perinatology, University Medical Center Utrecht, Netherlands
[2]Gynecology and Obstetrics, Rijnstate Hospital, Arnhem, Netherlands
[3]Laboratory for Neuroimmunology, University Medical Center Utrecht, Netherlands
[4]Pathology, University Medical Center Utrecht, Netherlands

Background Antenatal glucocorticoid (GC) treatment reduces mortality and morbidity of the preterm neonate. Actions of GCs are mediated by corticosteroid receptors, which are highly expressed in the hippocampus, a brain structure involved in cognitive functions. Studies in rodents have shown that exposure to therapeutic doses at critical developmental stages, reduces hippocampal volume and the number of hippocampal neurons shortly after treatment. Follow-up at adulthood showed adverse effects on memory and spatial learning (1).

Aim To investigate if antenatal GC treatment has an effect on hippocampal histology of the human preterm newborn.

Methods Patients included were neonates with a gestational age between 24 and 32 weeks, born between 1991 to 2009, who had died within 4 days after delivery and underwent a brain autopsy. Excluded were neonates with congenital malformations and neonates treated postnatally with GCs. Samples of the hippocampus were stained with H&E. The density of large and small neurons in the hippocampus was scored using semi quantitative analysis: high (4 points), moderate (3 pts), low/moderate (2 pts) or low (1 pt). Neuronal density irrespective of size was calculated adding the score of small and large neurons. Scoring was performed with the observer blind to the treatment group.

Results From 22 neonates the hippocampus was available for histological examination. Eleven had received a course of antenatal GCs, consisting of two i.m. doses of 12 mg betamethasone. No significant differences between the two groups were found regarding gestational age at delivery, birth weight and birth weight percentile. The hippocampus of neonates

who had been treated with antenatal GCs showed a lower density of neurons irrespective of size and of large neurons as compared to the control group (both P<0.05). No difference was found in density of small neurons.

Conclusion We found a significantly lower density of neurons in the hippocampus of neonates after antenatal glucocorticoid treatment.

(1) Noorlander CW et al. Dev Neurobiol. 68(2): 237-246, 2008

003

THE EFFECTS OF HUMAN AMNION EPITHELIAL CELLS (HAECS) ON FETAL BRAIN INJURY INDUCED BY LIPOPOLYSACCHARIDE

T. Yawno, J. Schuilwerve, T. Moss, P. Vosdoganes, A. Westover, G. Jenkin, E. Wallace, S. Miller

The Ritchie Centre, Monash Institute of Medical Research, Monash University, Clayton, VIC, Australia

Background: Intrauterine infection is recognized as a major cause of preterm labour and complications associated with neurodevelopmental changes. This study aims to examine whether human Amnion Epithelial Cells (hAECs) can be used as a potential therapeutic agent to reduce brain injury induced by intra-amniotic administration of Lipopolysaccharide (LPS) in preterm fetal sheep.

Methods: Pregnant ewes underwent surgery at ~110 days of gestation (term is ~147d) for implantation of catheters into the amniotic cavity, fetal trachea, carotid artery and jugular vein. LPS was administered at 117d; hAECs were labeled with CSFE and administered at 0, 6 and 12 hours into the fetal jugular vein, trachea or both. Control fetuses received an equivalent volume of saline. Brains were collected 7 days later, for histological assessment of brain injury.

Results: hAECs were found throughout the brain, with large numbers identified in the periventricular white matter, subcortical white matter, external capsule and the hippocampus and fewer in the cerebellum. Double-labeling showed that hAECs were co-localised with astrocytes (GFAP positive). Microglia and macrophages were present in focal and diffuse patterns in fetuses that received LPS, indicative of inflammation. Inflammation was reduced in fetuses that received hAECs via the jugular vein. In LPS fetuses, the number of TUNEL positive cells was significantly elevated in the white matter (>40 cells/region), compared with controls, and reduced in fetuses that received hAECs via the jugular vein (10-20 cells/region) and jugular vein+trachea (10-20 cells/region), but not in fetuses that received cells via the trachea alone (>40 cells/region). Pyknotic cells were found in the Purkinje cell layer in LPS treated brains (0.24 ± 0.1 cells/μm) and were reduced in fetuses that received hAECs via the jugular vein (0.06 ± 0.02 cells/μm), trachea (0.07 ± 0.05 cells/μm) and both (0.06 ± 0.02 cells/μm).

Conclusion: hAEC administration to fetuses in an LPS model of chorioamnionitis reduces inflammation and white matter injury.

004

EFFECT OF INTRAUTERINE INFLAMMATION ON FETAL AND NEONATAL CARDIOPULMONARY AND CEREBRAL CIRCULATION.

R. Galinsky[1], G. R. Polglase[1,2], S. B. Hooper[1,2], T. J.M. Moss[1,2]

[1]The Ritchie Centre, Monash Institute of Medical Research, Melbourne, VIC, Australia

[2]Department of Obstetrics and Gynaecology, Monash University, Melbourne, VIC, Australia

Background: Intrauterine inflammation is an antecedent of preterm birth and is often accompanied by a systemic fetal inflammatory response, which affects the cerebral and pulmonary vasculature. We examined the effect of antenatal inflammation on pre- and post-natal cardiopulmonary and cerebral circulations. Method: At ~112 days of gestation (d: term is ~147d) fetal surgery was performed for implantation of arterial and venous catheters and Transonic flow probes. At ~118 d, inflammation was induced by intra-amniotic administration of E coli lipopolysaccharide (LPS; serotype 055:B5; 20 mg); fetal pulmonary and cerebral blood pressures and flows were monitored for 7 days. Fetuses exposed to LPS (n=5) or saline (n=5) were delivered at ~125 d and mechanically ventilated for 40 minutes; arterial pressures and flows were monitored throughout the post-natal period. Results: Brachiocephalic arterial pressure was reduced in LPS-exposed fetuses relative to control (p<0.05). Fetal carotid and pulmonary blood flows, and pulmonary arterial pressure were not different. During the post-natal period pulmonary blood flow was initially lower than control after birth (p<0.05 at 5 minutes) and pulmonary vascular resistance was higher than control (p<0.05). Neonatal brachiocephalic and pulmonary arterial pressures, and carotid blood flow, did not differ in LPS-exposed preterm lambs relative to control. However, cerebral oxygen delivery was higher in LPS-exposed preterm lambs compared to control (interaction p<0.05). Conclusions: Intrauterine inflammation reduces fetal brachiocephalic arterial pressure; this does not persist into the neonatal period in preterm lambs. Following premature birth, intrauterine inflammation reduces pulmonary blood flow and increases pulmonary vascular resistance. The observed changes to fetal and neonatal circulations following intrauterine inflammation are likely due to structural remodelling of the vasculature.

005

DOPAMINE IS INEFFECTIVE FOR CARDIOVASCULAR SUPPORT AFTER ASPHYXIA IN NEAR-TERM FETAL SHEEP

P. P. Drury[1], L. Bennet[1], L. C. Booth[1,2], B. Wibbens[1], A. J. Gunn[1,3]

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[2]Howard Florey Institute, University of Melbourne, Melbourne, VIC, Australia
[3]Starship Children's Hospital, Auckland, New Zealand

Background: Dopamine is both a positive inotrope and vasopressor, and is commonly used for blood pressure support in the neonate. Surprisingly, there is limited empirical evidence to support its use. We hypothesised that following a near-terminal asphyxial insult cardiovascular support with dopamine would prevent secondary hypotension. Methods: 14 chronically instrumented near-term fetal sheep (120-126 days ga, term is 147 days) received complete umbilical cord occlusion for 15 min. Following release of occlusion if heart rate was not above 100 bpm or mean arterial pressure (MAP) not above 20 mmHg within 30 s fetuses were given adrenaline (0.1-0.3 ml/kg of 1/10 000 adrenaline). Dopamine infusion was initiated if MAP fell below 90% of baseline values within 6 h after reperfusion, starting at 4 µg/kg/min and titrated according to MAP up to 20 µg/kg/min. Results: 4 fetuses died during the initial reperfusion despite adrenaline administration. 10 fetuses survived beyond the initial reperfusion phase, of which 5 received adrenaline. 6 fetuses did not require dopamine. 4 fetuses required dopamine beginning at 96, 168, 180 and 264 min after occlusion. Dopamine was associated with a transient increase in MAP and fall in femoral blood flow. Despite increasing dopamine infusion to the maximal dose terminal hypotension developed in all four fetuses. Conclusion: Dopamine infusion is an ineffective strategy for cardiovascular support following asphyxia in near-term fetal sheep. Further work is required to assess putative agents for blood pressure support.

006

XANTHINE OXIDASE-DERIVED REACTIVE OXYGEN SPECIES AFFECT CARDIOVASCULAR FUNCTION IN THE FETUS

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The fetal circulation is under neural, endocrine and local control (Giussani *et al., Fetal Mat Med Rev* 6:17, 1994). We have also shown that the balance between reactive oxygen species (ROS) and nitric oxide (NO) provides an oxidant tone that regulates fetal femoral and umbilical blood flow (Thakor et al., *J Physiol* 588:4235, 2010; Thakor et al., *J Pineal Res* 49(4):399, 2010). However, the source of ROS in the fetal vasculature under basal or stimulated conditions is unknown. Using chronically-instrumented pregnant sheep in late gestation, this study tested *in vivo* the hypothesis that xanthine oxidase (XO)-derived ROS interact with NO to alter fetal cardiovascular control during basal and hypoxic conditions.

METHODS: Pregnant sheep and their fetuses (n=12; 0.8 gestation) were surgically prepared with vascular catheters and a fetal femoral and umbilical Transonic flow probes. After 5d, mothers were infused for 30 min with vehicle or allopurinol (150mg.kg^{-1} i.v.) or allopurinol following fetal NO synthase (NOS) blockade (250mg L-NAME with 5.1±2.0 mg.kg^{-1}.min^{-1} sodium nitroprusside i.v.) This technique permits *in vivo* blockade of fetal NOS while maintaining basal cardiovascular function (Gardner and Giussani. *Circulation* 108:331, 2003). Plasma uric acid, the end-product of the XO pathway was measured. 100min following infusion, fetuses were subjected to either i.a. bolus doses of phenylephrine (5-50µg; n=6) or a 30 min hypoxic challenge (fetal P_aO_2≈10mmHg; n=6).

RESULTS: During basal conditions, maternal allopurinol decreased fetal uric acid, it increased baroreflex gain and umbilical vascular conductance and diminished the fetal femoral vasopressor responses to phenylephrine. During hypoxia, maternal allopurinol prevented the rise in fetal uric acid and it diminished the fetal femoral vasoconstriction. The effects of maternal allopurinol on femoral vascular reactivity and on baroreflex gain were restored during fetal NOS blockade.

CONCLUSION: XO-derived ROS interact with NO to alter cardiovascular control in the fetus.

Supported by The BHF, The Elmore and Baird Funds and The Royal Society

007

DIFFUSE WHITE MATTER INJURY OF PREMATURE BRAIN IN THE CHRONICALLY INSTRUMENTED FETAL SHEEP

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[2]Obihiro University of Agriculture and Veterinary Medicine, obihiro,hokkaido, Japan

Background.

The pathogenesis of diffuse white matter injury in premature babies is poorly understood. Cerebral ischemia and intrauterine inflammation are thought to be associated.

Objectives.

To determine the effect of cerebral ischemia and/or intrauterine inflammation on the development of oligodendroglial lineage in cerebral white matter.

Methods.

Chronically instrumented fetal sheep was induced hemodynamic insult (H group; n=5), inflammation (I group; n=5), or inflammation+hemodynamic insult (I+H group; n=8). Sham operation was performed on control group (n=6). In H group, ~35% of fetoplacental blood volume was withdrawn at 110 days of gestation (DG). In I and I+H group, inflammation was induced by daily administration of granulocyte colony stimulating factor (G-CSF; 40 m g/day, 105-109 DG) and intra-amniotic injection of endotoxin (20mg, 107 DG), where hemorrhage or exchange transfusion (~35% of fetoplacental blood volume) was added at 108 DG in I+H group only. The brain was collected at 113 DG. Gross anatomical analysis was performed on H&E stain. TUNEL stain and immunohistochemistry of NG2, O4, CNPase, and PLP were performed and integrated density (ID) of each stain was statistically compared.

Results.

Periventricular leukomalacia was seen in all fetuses of I+H and H group. In I and I+H group, ID of O4 was significantly lower than that in control, whereas ID of CNPase was significantly higher. In H group, ID of O4 was significantly lower than that in control, while ID of PLP was higher. ID of NG2 showed no significant difference in all groups. The cell density of TUNEL in each treatment group was significantly lower than that in control.

Conclusion.

The current study suggests that in the premature fetus, cerebral ischemia and/or intrauterine inflammation accelerates the oligodendroglial maturity from precursor cells to immature cells rather than induces apoptosis of precursor cells in the cerebral white matter.

008

SYSTEMIC INHIBITION OF EXTRACELLULAR SIGNAL-REGULATED KINASE (ERK) HAS A PROTECTIVE EFFECT IN NEONATAL CEREBRAL WHITE MATTER INJURY

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Centre for Perinatal Brain Protection and Repair, Institute for Women's Health, University College London, LONDON, Great Britain

*Backround:*The ERK cascade, a major component of mitogen-activated protein kinase signalling, is important in synaptic plasticity, mediating mitogenic and trophic effects, and cell proliferation in normal and transformed non-neuronal cells. Previous studies implicate the activation of axonal ERK as an important component of neuronal cell death following perinatal hypoxia-ischaemia (HI). The aim of this study was to investigate the effects of global and neuron-specific ERK-inactivation, using pharmacological ERK inhibitors and cell-specific mouse mutants, on markers of neural cell damage following HI insult. *Methods:*HI was induced in postnatal day 7 C57/Bl6 mice (approx 31-32 week human gestation) using the Rice-Vanucci model followed by 30min (moderate) or 60min (severe) hypoxia (8%O2/N2). The control groups were littermate pups that were either sham operated or left naïve. The mice were sacrificed at given time points up to 96h post HI. Forebrain damage was assessed through extent of cell death (TUNEL), neuronal loss (Nissl) and levels of microglial activation (AlphaM). SL327, a competitive Mek 1/2 inhibitor, was injected 20min prior, 0min or 60min after HI. In order to assess the role of neuronal ERK in neonatal HI, we used neuron-specific ERK mutant mice combining global ERK1-deletion and floxed ERK2-gene, controlled by Synapsin-driven Cre-recombinase (S1122). *Results:*pERK was detected in periventricular white matter (WM) axons (15-45min post HI), followed by white and grey matter (GM) glia and cortical neurons (1-4h post HI), returning to normal by 8h. SL327-intervention ablated pERK-expression by 100% in WM and GM, significantly reducing cell death (50-60%) and alphaM (50%) in GM-regions ($p<0.05$, t-test). AlphaM levels also decreased in WM (50%,$p<0.05$). S1122 mutants replicated pERK-disappearance through SL327, however, TUNEL and alphaM increased in WM (50%, 250% respectively, $P<0.05$) suggesting cell-specific response. *Conclusion:*These data suggest activated ERK as a promising target for therapeutic intervention in neonatal brain damage, and prevention of periventricular leukomalacia.

009

MELATONIN AS A NEUROPROTECTANT IN THE GROWTH RESTRICTED OVINE FETUS FOLLOWING GLUCOCORTICOID ADMINISTRATION

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Intrauterine growth restriction (IUGR) is associated with fetoplacental hypoxemia and increased incidence of neurological morbidity and mortality. The IUGR fetus has an increased risk of preterm birth therefore these 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.

Time mated pregnant ewes carrying twins underwent surgery at 105-110 days gestation (term ~147d). In one fetus we carried out single umbilical artery ligation (SUAL) to induce IUGR and the other fetus acted as an age-matched control. Each twin was implanted with a flow probe around the carotid artery and femoral artery catheter for sampling. Betamethasone (BM; 11.4mg i.m. to ewe) or vehicle was given on days five (BM1) and six (BM2) following surgery with melatonin (MLT; 2mg bolus, 2mg/hr i.v. to ewe) administration commencing 30 minutes prior to the first dose. Post mortem was conducted on day seven; the fetal brain was fixed and processed for light microscopy.

Compared to basal values, carotid blood flow does not change over time in animals that did not receive BM or in control+BM or control+BM+MLT fetuses ($p>0.05$). At 14hrs post BM1 carotid blood flow is significantly increased in both SUAL+BM (49.5±15.9% increase, $p<0.001$) and SUAL+BM+MLT (62.2±21.9% increase, $p=0.009$) fetuses. A similar increase occurs 15hrs post BM2 (SUAL+BM 33.3±15.6% increase, $p=0.003$; SUAL+BM+MLT 75.8±23.8% increase, $p=0.001$). Preliminary data within the brain suggest that the number of 4-HNE (lipid peroxidation) positive cells is increased in the periventricular white matter of SUAL+BM fetuses however exposure to melatonin reduced 4-HNE staining.

Melatonin does not prevent the rebound carotid blood flow reperfusion that occurs in IUGR fetuses exposed to maternal betamethasone, however melatonin does appear to protect against oxidative stress within the fetal brain.

010

MATERNAL DIET SUPPLEMENTATION WITH CREATINE FROM MID-GESTATION PROTECTS THE NEWBORN SPINY MOUSE BRAIN FROM BIRTH HYPOXIA.

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[3]Centre for Physical Activity and Nutrition Research, Deakin University, Melbourne, VIC, Australia

Background: The creatine-phosphocreatine shuttle is essential for the maintenance of cellular ATP, particularly under hypoxic conditions when respiration may become anaerobic. Using a model of intrapartum hypoxia in the precocial spiny mouse (*Acomys cahirinus*), the present study assessed the potential for maternal creatine supplementation during pregnancy to protect the developing brain from the effects of birth hypoxia.

Methods: On day 38 of gestation (term 39 days), the pregnant uterus was isolated and placed in a saline bath for 7.5 min, inducing global hypoxia. The pups were then removed, resuscitated, and cross-fostered to a nursing dam. Control offspring were delivered by caesarean section and recovered immediately after release from the uterus. 24 hours after delivery, pups were weighed and killed. The cerebellum and brainstem were removed, and the cerebrum snap frozen for use in the lipid peroxidation assay, or whole brains were immersion-fixed in 10% phosphate-buffered formalin for 24-36 h, embedded in paraffin and coronal sections cut (5μm) from bregma 2.06 to 2.30 mm for immunohistochemistry.

Results: At 24 h after birth hypoxia, the brains of offspring from dams fed a normal diet showed significant increases in lipid peroxidation as measured by the amount of malondialdehyde. In the cortical subplate, thalamus and piriform cortex there were significant increases in cellular expression of the pro-apoptotic protein BAX, cytoplasmic cytochrome-c and caspase-3. When pregnant dams were fed the creatine supplemented diet, the increase in malondialdehyde, BAX, cytochrome-c and caspase-3 were almost completely prevented, such that they were not different from control (caesarean-delivered) neonates.

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

011

CEREBRAL MYELINATION IN IUGR: NOT JUST MYELIN BASIC PROTEIN.

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Background: Intrauterine growth restriction (IUGR) can lead to adverse neurodevelopmental sequelae in postnatal life. Our objective was to determine whether IUGR, induced by CPI in the guinea pig results in long-term deficits in brain myelination and could therefore contribute to altered neural function.

Methods: At 30dg, CPI was induced in pregnant guinea pigs via uterine artery ligation (UAL) to produce IUGR fetuses (60dg), neonates (1 week) and young adults (8 weeks); controls were from the un-ligated horn or sham-operated animals. At 60dg (control, n=8 and IUGR, n=8), 1 week (control, n=7 and IUGR, n=7) and 8 weeks (control, n=12 and IUGR, n=12) of age the brains were perfused and processed for thionin-staining and immunohistochemistry using antibodies for myelin basic protein (MBP), myelin-associated glycoprotein (MAG), myelin-proteolipid protein (PLP) and oligodendrocyte transcription factor 2 (Olig2). White matter (WM) and cortical volume, MAG- and Olig2-immunoreactive (IR) oligodendrocyte density and the extent of myelination were determined using image analysis in the cerebral hemispheres.

Results: In IUGR fetuses and neonates, WM volume was reduced ($p<0.05$); this reduction did not persist in young adults however the corpus callosum width was reduced ($p<0.05$). Immunoreactivity for MBP, MAG and PLP, all markers of early myelinating oligodendrocytes, was reduced in IUGR fetuses compared to controls. Of these markers MBP was the most markedly affected with an abnormal retention of protein in the oligodendrocyte soma and a reduction of its incorporation into the myelin sheath. MAG-IR oligodendrocyte density was reduced ($p<0.05$), while Olig2-IR oligodendrocyte density was increased ($p<0.05$). MBP-, MAG- and PLP-IR recovered to control levels postnatally.

Conclusions: These results suggest that IUGR transiently delays oligodendrocyte maturation and myelination *in utero* but that myelination and WM volume are restored postnatally. Although myelination reaches control levels postnatally, a maturational delay might affect normal functional development of the nervous system.

012

SLEEP ARCHITECTURE OF PRETERM FETAL SHEEP AND THE EFFECT OF ANTENATAL GLUCOCORTICOIDS

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Background: Perinatal EEG monitoring is increasingly being used to provide rapid diagnostic and prognostic information to help facilitate treatment. However, there is a need to understand the impact of adverse events as well as standard treatments on brain activity. Antenatal glucocorticoids have been reported to impair brain development, and thus may alter EEG maturation. We examined the acute and chronic effects of dexamethasone on preterm fetal sleep architecture.

Methods: Ewes at 103d gestation, received two intramuscular injections of either dexamethasone (DEX, 12mg/2ml, n=8) or saline vehicle (2ml, n=7) 24h apart (DEX-1 and DEX-2). Fetal EEG activity was monitored continuously before and until 120h after the first injection.

Results: In control fetuses sleep architecture was discontinuous and characterised by mixed amplitudes and frequencies, transient waveforms, and periods of quiescence (interburst intervals (IBI)). Over time there was a decrease in the percentage of time spent and amplitude of delta frequency, an increase in theta, alpha and beta activity, and a fall in the number and duration of IBI. All frequency bands and IBI displayed a diurnal rhythm. Dexamethasone caused an acute increase in the percentage and amplitude of delta, and a fall in the other frequencies. Over the longer term there was a significant rise in theta, alpha and beta frequencies, and a tendency for reduced delta. Numbers of transient waveforms fell significantly, as did IBI duration and number of events, and there was a loss of IBI diurnal rhythm.

Discussion: DEX administration appears to enhance neural maturation in preterm fetal sheep, with a switch to higher frequencies and a reduction in IBI. This may reflect maturation in excitatory synapses and loss of subcortical gating of the factors which inhibit cortical activity creating the IBI quiescence. These findings highlight the importance of studying the effects of common treatments on the EEG activity.

013

MATURATION OF MITOCHONDRIAL REDOX RESPONSE TO PROFOUND INTRAUTERINE HYPOXIA

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Background: The fetal brain becomes progressively more vulnerable to hypoxic damage in the last third of gestation. In this study we examined the hypothesis that this is associated with impaired mitochondrial adaptation to profound hypoxia. Methods: Chronically instrumented fetal sheep at 0.6, 0.7 and 0.85 gestation (ga) were subjected to either 30 min (0.6 gestational age (ga), n=6), 25 min (0.7 ga, n=22) or 12-15 min (0.85 ga, n=10) of complete umbilical cord occlusion. Near-infrared spectroscopy (NIRS) derived intra-cerebral oxygenation (DHb = HbO2 – Hb), total haemoglobin (THb) and cytochrome oxidase (CytOx) redox state were monitored continuously. Results: After occlusion THb initially increased significantly at 0.6 and 0.7 ga, to a maxima at 7 min, whereas there was no change at 0.85 ga ($p<0.05$). From 7 min THb then fell in all groups. DHb initially fell rapidly in all groups to a plateau from 6 min. CytOx initially increased in all groups with the greatest rise at 0.85 ga ($p<0.05$). Strikingly, the 0.85 group showed a progressive fall after 5 min of occlusion, whereas immature fetuses showed a sustained rise for the remainder of the occlusion periods ($p<0.05$). Conclusions: The rapid rise in oxidized CytOx in parallel with loss of oxygenated haemoglobin after occlusion denotes reduced electron flow down the mitochondrial electron transfer chain due to loss of oxidative metabolism. The greater and more rapid initial rise in CytOx after the start of occlusion in near-term fetuses denotes greater fixed dependence on oxidative metabolism. In contrast, the late loss of oxidized CytOx near-term is unexplained but speculatively may be mediated by inhibition of cytochrome activity by nitric oxide or by opening of the mitochondrial pores, favouring programmed cell death.

014

VULNERABILITY OF IUGR PREGNANCIES TO PRETERM LABOUR: ACTIVATION OF INFLAMMATORY PROSTAGLANDIN PATHWAY

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Background: Intrauterine growth restriction (IUGR) is a common risk factor of preterm labour; however, the mechanisms of the relationship remain unknown. Prostaglandins (PGs) are key downstream stimulants of parturition including membrane rupture, cervical dilatation and uterine contractions. PG availability is regulated by the synthetic enzyme prostaglandin H synthase (PGHS) and the metabolising enzyme 15-hydroxyprostaglandin dehydrogenase (PGDH). We hypothesised that IUGR increases susceptibility to preterm labour due to increased PG availability caused by the changing balance of PG synthetic and metabolising enzymes in intrauterine tissues.

Methods: Myometrium, amnion, chorion and placentae were collected from sham operated and surgically induced IUGR pregnancies throughout late gestation (>62days). IUGR was classified as brain to liver ratios >0.9. PGHS-1 and PGDH protein expression were quantified by western blot. Data were analysed by two way ANOVA and post hoc analyses.

Results: Our model of IUGR in the guinea pig results in a significantly shorter gestation. IUGR significantly upregulated myometrial PGHS-1 expression compared to controls over late gestation ($P<0.05$). Chorionic PGDH expression was markedly decreased ($P<0.002$) in IUGR pregnancies. This difference persisted when data was further analysed by days prior to expected term, with IUGR pregnancies expressing significantly lower levels of PGDH a week prior to labour ($P<0.002$).

Conclusion: These findings suggest a preterm activation of the inflammatory PG cascade in IUGR pregnancies as demonstrated by an upregulation of myometrial PGHS-1 and a marked suppression of chorionic PGDH. This increase in prostaglandin availability may contribute to the vulnerability of IUGR pregnancies to preterm labour.

015

DEVELOPMENT OF AN ARTIFICIAL PLACENTA : PUMPLESS ARTERIOVENOUS EXTRACORPOREAL LIFE SUPPORT IN A PREMATURE LAMB MODEL

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Objectives. Effective treatment for premature low-birth-weight infants with cardiopulmonary abnormalities remains an unsolved problem. The development of an artificial placenta is an appealing alternative. The purpose of this study was to develop an artificial placenta in the form of a pumpless arteriovenous extracorporeal life support circuit that could maintain fetal circulation. Methods. Lambs (n=8) were delivered by caesarian section between 125 and 135 days of gestation (term 147 days). After exteriorizing the fetuses, 2 catheters connected one side to an artificial placenta (membranous device for gas exchange) were placed the other side into umbilical artery and vein. Just after cutting off the umbilical cord, fetuses were submerged in a warm saline bath. Fetal heart rate, arterial pressure, central venous pressure, and blood flow of umbilical circuit were continuously monitored and blood gas analysis was examined. Dopamine was infused to maintain mean arterial pressure (MAP) and cardiac performance assessed by echocardiography. Organ blood flow was measured with colored-microsphere technique. Results. Three of eight fetuses survived for more than 14 hours with the artificial placenta while maintaining fetal circulation. Total flow of the artificial placenta circuit (30~120 ml/kg/min) correlated with MAP ($p < 0.05$, Spearman correlation coefficient 0.75) and arterial oxygen content ($p < 0.05$, Spearman correlation coefficient 0.51). Dopamine could increase MAP transiently. However, blood lactate was increased and the cardiac performance was gradually deteriorated over time even while the gas exchange of the artificial placenta was maintained. Following 2~6 hours of age, the cerebral blood flow appeared to start decreasing. Conclusion. Elevated MAP in our experiment might be achieved by systemic vasoconstriction, which led to decreased systemic organ flow due to increased artificial placental flow, resulted in systemic circulatory failure. It may be important to retain both systemic and circuit circulation by controlling each resistance.

016

ROLES OF THE PLACENTAL RENIN-ANGIOTENSIN SYSTEM IN PLACENTAL VASCULOGENESIS?

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A placental renin-angiotensin system (RAS) could be involved in placental development. Prorenin binds to the (pro)renin receptor ((P)RR) and can generate angiotensin I (Ang I) from angiotensinogen (AGT), which is converted to Ang II by angiotensin-converting enzyme (ACE). Ang II acts via the type 1 or type 2 angiotensin receptors (AT_1R or AT_2R). ACE2 terminates the action of Ang II by converting it to Ang 1-7.

We examined the expression and localization of the RAS as well as the angiogenic factor, vascular endothelial growth factor (VEGF) in early gestation (6-16 weeks) placentae collected from women undergoing elective termination of pregnancy (n=33) or from women delivering by elective caesarean section at term (>37 weeks, n=10), respectively. Prorenin, (P)RR, AGT, ACE1, ACE2, AT_1R, AT_2R and VEGF mRNAs were measured by qPCR. Immunohistochemistry to localize RAS proteins was performed.

Prorenin mRNA levels were highest at 6-9 weeks ($P<0.02$) and lowest at term ($P<0.03$). (P)RR mRNA levels were also lowest in term placentae ($P=0.000$). Levels of VEGF and AT_1R mRNA were not altered throughout gestation however, renin, (P)RR and AT_1R mRNA levels were highly correlated with VEGF mRNA abundance ($P=0.000$, $r=0.606$; $P=0.000$, $r=0.703$, and $P=0.001$, $r=0.478$, respectively). AGT mRNA was low in all samples.

In early gestation placentae prorenin and AT_1R protein were localized to the cytotrophoblasts (CTBs), syncytiotrophoblast (STBs) and extravillous trophoblast cells (EVTs), whereas (P)RR protein was localized to the STB and EVTs, but not CTBs. ACE2 was also localized to STB and CTB whilst ACE was only localized to the fetal endothelium. AGT protein was localized to trophoblasts.

Therefore, the expression of prorenin and (P)RR mRNA was highest in very early gestation placentae when vasculogenesis is maximal. As they and AT_1R were highly correlated with VEGF this is the first evidence that the placental RAS may regulate placental angiogenesis.

017

ROLE OF ACTIVIN A IN THE PATHOPHYSIOLOGY OF PREECLAMPSIA

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Background: Preeclampsia (PE) is a pregnancy specific disorder characterized by sudden onset of hypertension and proteinuria. There is increasing evidence to suggest that oxidative stress plays a profound role in the pathophysiology of preeclampsia. Aim: Determine role of Activin A in induction of oxidative stress. Hypothesis: Activin A is a key molecules involved in the pathophysiology of PE and is directly related to the oxidative stress. Results: Activin A directly increased production of reactive oxygen species (ROS) in HUVECs (***p<0.001). This was mitigated with the addition of follistatin 288 (FS288), SOD, tempol or apocynin. Activin A also increased 8-isoprostane levels in a dose-dependent manner (*p<0.05). Similarly, this effect was mitigated through the addition of FS288 and all antioxidants tested. A similar trend was observed when HUVECs were tested with 20% PE serum, FS288 and antioxidants. Following stimulation with activin, gene expression of ZO-1 was reduced by 50% (**p<0.01), ET-1 expression increased >5-fold (**p<0.01), Nox 2 expression increased ~4-fold (*p<0.05). Both transendothelial permeability and resistance assays showed that there was a reduction in endothelial integrity when HUVECs were exposed either activinA or PE serum (*p<0.05). This was restored with the addition of FS288 or apocynin. Reduction in endothelial resistance by activin A and PE serum (**p<0.01) was prevented following knockdown of Nox2. Conclusion: We present a novel concept in treatment of preeclampsia. Limiting the bioavailability of activin A or reduction of oxidative stress is likely to augment widespread maternal endothelial dysfunction seen in preeclampsia.

018

VASODILATOR EFFECT OF HYDROGEN SULPHIDE (H_2S) IN THE PERFUSED HUMAN PLACENTA

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Background: Alongside nitric oxide and carbon monoxide, novel H_2S is the third gaseous signalling transmitter. Endogenous H_2S production is catalysed by cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) through desulphydration of cysteine[1,2]. H_2S can hyperpolarise cell membranes by targeting KATP channels3, and thus relax smooth muscle cells to act as a vasodilator. However, the role of endogenous or exogenous H2S has not been studied in the fetoplacental circulation or complications of pregnancy. Methods: Perfusion pressure and flow (Transonic Inc.) were measured in 8 Caesarean-delivered human placentae. Single lobes were perfused with equilibrated (95% O2/5% CO2, pH 7.4) Earle's bicarbonate buffer by cannulating the chorionic artery and vein. Following pre-constriction with the thromboxane mimetic U46619 (10-7mol/L), increasing NaHS (10-12 to 10-6mol/L) was infused. Glybenclamide (10-5mol/L) was administered to block KATP</sub> channels, and L-NAME (10-5mol/L) to inhibit endogenous nitric oxide synthesis. Protein and RNA were extracted from 6 Caesarean-delivered, 6 pre-eclamptic and 6 IUGR placentae to determine the expression of CBS and CSE under normal and pathological conditions. Results: H2S donor NaHS led to significant concentration-dependent reductions in perfusion pressure and vascular resistance. The vasodilator effect of NaHS (AUC arbitrary units: 397±7) was diminished by L-NAME (332±6) and further reversed by glibenclamide (266±5; P<0.05). The primary vasodilator actions of H2S in the human placenta are thus mediated via KATP</sub> channels, and an additional interaction between H2S and NO exists. IHC confirmed CBS and CSE in the syncytium and vascular smooth muscle cells, respectively. CBS protein and RNA levels remained unchanged, whilst CSE was down-regulated in pre-eclamptic and IUGR placentae. Conclusion: We show for the first time that H2S is a potent vasodilator in the human placenta and that enzymes responsible for its synthesis are down-regulated in pathological placentae associated with impaired placental blood flow and fetal growth restriction. (EAH & TCD contributed equally, DAG is corresponding author)

(1) Bukovska G et al. Expression of human cystathionine beta-synthase in Escherichia coli: purification and characterization. Protein Expr Purif 5, 442-8, 1994.
(2) Erickson PF et al. Sequence of cDNA for rat cystathionine gamma-lyase and comparison of deduced amino acid sequence with related Escherichia coli enzymes. Biochem J 269, 335-40, 1990.
(3) Reiffenstein RJ et al. Toxicology of hydrogen sulfide. Annu Rev Pharmacol Toxicol 32, 109-34, 1992.

019

EPIGENETIC REGULATION OF INFLAMMATORY GENES IN THE AMNION DURING PREGNANCY

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Increased expression of inflammatory genes in the fetal membranes is essential for parturition, but the mechanism programming the increase for the end of gestation is unknown. Epigenetic chromatin modifications influence gene activity independently of DNA sequence information and are critical for establishing cell type-specific and developmental patterns of gene expression. We hypothesised that inflammatory genes in the fetal membranes are programmed by epigenetic mechanisms for increased expression at term. To test the hypothesis, we have determined the methylation density of CpG islands (a repressive epigenetic DNA modification) in the promoter of the inflammatory genes prostaglandin endoperoxide synthase-2 (PTGS2), bone morphogenetic protein-2, pre-B-cell colony stimulating factor and chemokine-CXC-motif ligand-2 by customised Methyl-Profiler assays in amnion samples from early (11-18 weeks) and term gestations and after term labour (n=8). Further, we used chromatin immunoprecipitation to determine the levels of the activating epigenetic histone modifications histone-3 lysine-4 trimethylation (H3K4me3), histone-3 acetylation (H3ac) and histone-4 acetylation (H4ac) and the level of the repressive epigenetic modification histone-3 lysine-27 trimethylation (H3K27me3) at the PTGS2 promoter in amnion tissue from early and term gestations (n=2-3). We found that CpG island methylation density varied widely between genes and among individuals, but did not change significantly with gestational age and labour. On the other hand, the level of the activating histone marks H3K4me3 and H3ac increased and the level of the repressive H3K27me3 mark decreased at the PTGS2 promoter at term vs. early gestation. The CpG island methylation data suggest that this epigenetic modification does not play a significant role in inflammatory gene expression programming during pregnancy. Histone modifications, however, appear to poise the PTGS2 gene for expression at term in preparation for labour. This is the first time that the activation of a labour-associated gene by chromatin modifications is reported suggesting that gestational length is epigenetically programmed.

020

EICOSAPENTANOIC ACID (EPA) IS MORE EFFECTIVE THAN DOCOSAHEXANOIC ACID (DHA) IN INHIBITING LPS-INDUCED LIPID HYDROPEROXIDE PRODUCTION AND OXIDATIVE DNA DAMAGE IN THE PLACENTA

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Background: The omega-3 (n-3) fatty acids, EPA and DHA, exert anti-inflammatory effects. However, the relative action of each and concerns about their susceptibility to oxidative degradation remain unanswered. As the placenta is a major source of oxidative stress causally implicated in processes affecting the fetus and neonate , we investigated the effects of DHA and EPA on antioxidant defences, oxidative stress and pro-inflammatory cytokine production induced by lipo-polysaccharide (LPS) in placental explants.

Method: Placental explants (n=8) were pre-treated with DHA or EPA (1mM, 10mM and 100mM) prior to LPS (1ng) exposure or co-exposed to LPS. Total antioxidant capacity (TAC), malondialdehyde (MDA, lipid peroxidation) and 8-hydroxy-2-deoxy Guanosine (8-OH-dG, oxidative DNA damage) was measured by ELISA.

Results: Low concentration (1mM-10mM): EPA and DHA increased MDA and 8-OH-dG production compared to controls with EPA induced MDA and 8-OH-dG lower compared to LPS ($p<0.01$). Co-treatment with EPA+LPS decreased MDA and 8-OH-dG production ($p=0.01$), an effect not observed with DHA+LPS. Pre-treatment with DHA or EPA inhibited LPS induced MDA and 8-OH-dG production ($p=0.01$) with the reduction in 8-OH-dG greater for EPA ($p<0.05$). High concentration (100mM): For all experimental conditions oxidative stress was higher than LPS alone. The DHA and EPA mediated effects were not paralleled by alterations in TNFα and IFNγ production. LPS exposure resulted in reduced TAC. Pre-treatment with EPA and DHA prior to LPS was associated with greater TAC than LPS alone ($p<0.01$).

Conclusions: Pre-treatment with low dose n-3 fatty acids limited LPS induced oxidative stress and increased TAC, with high doses promoting oxidative stress. EPA exerted a greater protective effect, not mediated by alterations in pro-inflammatory cytokines or anti-oxidant defences. With evidence supporting beneficial effects of n-3 LCPUFA's on neonatal neuro-development and the incidence of inflammatory morbidities, characterisation of mechanisms through which these effects are mediated will accelerate their implementation into clinical practice.

021

ELUCIDATING THE ROLE OF PROSTAGLANDINS IN THE FETAL INFLAMMATORY RESPONSE

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Intra-amniotic (IA) injection of lipopolysaccharide (LPS) induces inflammation and causes profound increases in pulmonary surfactant in the lungs of preterm fetal sheep. Pulmonary effects of IA LPS may be mediated by prostaglandins (PGs), fundamental inflammatory mediators with established roles in fetal lung maturation. We have shown that IA LPS increases PGE2 in the amniotic fluid and fetal plasma, and that gene expression of PGH Synthase type-2 (PGHS-2) increases in the fetal lung 2 days after IA LPS. We aimed to block the IA LPS-induced increase in PGE2 with a PGHS-2 inhibitor (nimesulide) and examine the effects 2 days after LPS administration. Twenty-four pregnant ewes underwent surgery at ~112 days of gestation (term ~147d) for cannulation of the amniotic cavity, fetal trachea and a fetal carotid artery and jugular vein, and the maternal jugular vein. At ~117d, 12 sheep received a continuous maternal intravenous infusion of saline (2mL/hr) and a single injection of LPS (E. coli 055:B5; 20mg; n=6) or saline (n=6) into the amniotic sac. At ~117d, 12 sheep received a continuous maternal intravenous infusion of nimesulide (50mg/hr) and a single injection of LPS (n=6) or saline (n=6) into the amniotic sac. PGE2 concentrations were measured by radioimmunoassay in amniotic fluid and fetal plasma. Inflammation was assessed by counting CD45-positive cells in fetal lung, chorioamnion, umbilical cord and cotyledon samples collected at ~119d. Maternal nimesulide infusion prevented PGE2 increasing in response to IA LPS in the amniotic fluid and fetal plasma. Nimesulide significantly reduced the IA LPS induction of CD45-positive cells in the chorioamnion ($p=0.01$), umbilical cord ($p=0.03$) and cotyledon ($p=0.005$) to control levels. Nimesulide reduced CD45-positive cell numbers in response to LPS ($p=0.016$) to the control level in the upper lung lobe, and reduced inflammation in the middle and lower lobes, albeit not to control levels. Nimesulide prevented inflammation in the chorioamnion, umbilical cord and cotyledon, and reduces, but does not entirely prevent, the inflammatory response in the lungs.

(1) Moss et al. (2002) American Journal of Obstetrics and Gynecology 187:1059-1065.
(2) Kitterman et al. (1981) Journal of Applied Physiology 51:1562-1567.

022

HUMAN AMNION EPITHELIAL CELLS MODULATE THE PULMONARY STRUCTURAL AND INFLAMMATORY RESPONSES TO INTRAUTERINE INFLAMMATION IN FETAL SHEEP

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Background: Intrauterine inflammation alters fetal lung development and infants' risk of lung disease. Human amnion epithelial cells (hAECs) can mitigate inflammation and aid tissue repair. We hypothesised that hAECs would attenuate inflammation-induced changes in fetal lung development. Method: At 117 days' gestation (GA) instrumented sheep received intra-amniotic (IA) lipopolysaccharide (LPS from E coli 055:B5, 20mg). Human AECs were administered either to the fetal jugular vein (IV), trachea (IT) or both (IV+IT) at 0, 6 and 12 hours. Controls received IA saline and no hAECs. Lungs were collected at 124 days GA. Results: LPS injection resulted in pulmonary inflammation, and altered lung structure and function. Human AECs attenuated changes in lung compliance ($p<0.05$, IV+IT v LPS) and lung structure: tissue-airspace ratio ($p<0.05$, IV+IT v LPS), alveolar septal crest density ($p<0.001$, all groups v LPS). Despite elevated leukocyte numbers in the lungs of hAECs-treated fetuses, inflammatory cytokines were reduced (TNFα, $p<0.01$, all groups v LPS; IL-1β, $p<0.01$, IV+IT v LPS; IL-6, $p<0.01$, IV and IV+IT v LPS). Conclusions: Human amnion epithelial cells attenuate both the fetal pulmonary inflammatory response to experimental intrauterine inflammation and resultant changes in lung development.

023

LUNG DEVELOPMENT IN POSTNATAL RATS FOLLOWING MATERNAL VITAMIN D DEFICIENCY

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Background: Vitamin D (Vit D) deficiency has emerged as a significant health problem, with approximately 20% of Australian women being Vit D deficient. Epidemiological studies have implied that Vit D deficiency during pregnancy can lead to an increased risk of asthma in offspring, suggesting that lung development is affected by Vit D deficiency. Aim: To determine the effects of maternal Vit D deficiency on the structure of the small conducting airways (bronchioles) and lung parenchyma, and the pulmonary immune status of postnatal offspring. Methods: Sprague-Dawley rats were fed a Vit D deficient diet for 6 weeks prior to pregnancy and throughout pregnancy and lactation; controls were fed a normal diet. At necropsy, 28 days after birth, lungs were collected for structural and molecular analyses. In the lung parenchyma we measured the percentages of tissue and airspace and the mean linear intercept. In the bronchioles we measured the area of epithelium and smooth muscle in the outer wall; we also measured the number of bronchiolar-alveolar attachments. We used quantitative real-time PCR to determine the mRNA expression of surfactant protein (SP)-A, -B, -C and -D and the cytokines interleukin (IL)-2, -4, -5 and -13 in lung tissue. Results: Vit D deficient pups had significantly lower Vit D serum concentrations (6.4±0.6nmol/L) than controls (20.9±1.0nmol/L) and were 13% lighter at necropsy compared to controls. Compared to controls of the same gender, the number of bronchiolar-alveolar attachments was 54% lower in male Vit D deficient pups, and lung IL-5 mRNA levels were lower in female Vit D deficient pups. No significant differences were observed in any other parameter measured. Conclusions: Vit D deficiency during development impairs growth and has gender specific effects on lung development. Fewer bronchiolar-alveolar attachments in Vit D deficient males could facilitate airway narrowing in later life.

024

INHALATION OF HYPEROXIC GAS IN THE NEONATAL PERIOD: LONG-TERM PULMONARY IMPLICATIONS

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Background: Very preterm infants are born with immature lungs and often require respiratory support, which includes hyperoxic gas. Numerous studies have reported poor lung function in children and adults who were born very preterm and received respiratory support, suggesting altered development of the small conducting airways of the lung (bronchioles). Objective: We have previously shown in mice that neonatal inhalation of hyperoxic gas causes persistent alterations in bronchiolar development in early adulthood (P56d; 56d postnatal age). Our aim was to determine if these structural alterations persist to "middle-age" (P10mo), and alter lung function. Methods: Neonatal mice (C57Bl/6J) born at term were continuously exposed to hyperoxic gas (65% O2) from birth until postnatal day 7 (n=15), after which they lived in room air (21% O2) until P10mo. Controls breathed room air from birth (n=16). At P10mo, the structure of the bronchioles and lung parenchyma was analysed, and lung function was assessed in response to increasing does of methacholine. Bronchoalveolar lavage fluid (BALF) was collected and immune cells were enumerated. Results: Hyperoxia-exposed mice had significantly more bronchiolar smooth muscle and fewer alveolar-bronchiolar attachments than controls; they also had a greater mean liner intercept and airspace fraction. With increasing methacholine doses, hyperoxia-exposed mice tended to have a smaller increase in pulmonary resistance (p=0.083) and a smaller decrease in dynamic compliance (p=0.099) than controls. At the highest methacholine dose (20mg/ml) hyperoxia-exposed mice had significantly higher compliance than controls. The total number of immune cells in BALF was 90% greater than controls; >95% of these were macrophages. Conclusions: Neonatal inhalation of hyperoxic gas results in persistent structural alterations of the bronchioles and lung parenchyma at "middle-age", which likely contribute to altered lung function. Increased numbers of immune cells indicate low-grade chronic inflammation and could affect the ability of the lungs to clear pathogens.

025

THE POTENTIAL ROLE OF REPAIR GENES UROKINASE RECEPTOR AND METALLOTHIONEIN IN THE REPAIR OF LUNG STRUCTURE FOLLOWING VENTILATION INDUCED INJURY IN THE IMMATURE LUNG

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Background: Very preterm infants can require mechanical ventilation (MV) at birth, which often causes lung injury. Even brief MV can rapidly activate inflammatory and early response injury genes. To investigate mechanisms of lung injury and repair we have used a novel method of ventilating fetal sheep to induce injury in the immature lung, which resolves spontaneously within 15 days. Aim: To investigate mechanisms of injury and repair in the very immature lung during the canalicular and early alveolar stages of lung development after brief, injurious MV. We focussed on the potential role of early response genes, inflammatory genes and repair genes. Methods: Pregnant sheep underwent aseptic surgery to exteriorize the fetal head and chest at either 110 days of gestational age (GA, canalicular stage, n=6) or 125d GA (early alveolar stage, n=8, term ~147dGA). Fetuses were ventilated for 2h using an injurious ventilation protocol and then returned to the uterus. Lungs were collected 24h after MV and compared to unventilated controls. mRNA levels were measured by qRT-PCR. Protein deposition was determined by immunohistochemistry. Results: 24 hours after MV, metallothionein and urokinase receptor mRNA levels were significantly increased in canalicular (2.7+0.4 and 2.9+0.8 fold increase respectively) and early alveolar stage (2.6+0.7 fold and 1.7+0.3 fold increase respectively) lungs. There was no difference in the mRNA levels of the early response genes Connective Tissue Growth Factor, Cysteine Rich 61 and Early Growth Response 1 at either stage of lung development. Gene expression of pro-inflammatory cytokines Interleukins -1β, -6, -8 and Tumor Necrosis Factor-α were also not different at either stage of lung development. Nor were there differences in the protein abundance of EGR1 and CYR-61 at either stage of lung development. Conclusions: Lung repair processes commence with normalisation of early response and inflammatory gene expression and the activation of repair genes, within 24h of brief, injurious MV.

026

THE EFFECT OF ACUTE CAFFEINE ADMINISTRATION ON PULMONARY FUNCTION IN VENTILATED VERY PRETERM LAMBS.

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Background: Caffeine is commonly used in the clinical management of very preterm infants. A recent study demonstrated caffeine is associated with a reduction in chronic lung disease (CLD) and patent ductus arteriosus (DA) in very preterm infants (1), however the mechanisms involved are unknown. Our aim was to determine the effect of acute caffeine administration on pulmonary function in ventilated very preterm lambs. Methods: Catheterised fetal sheep, with Transonic flow probes around the left pulmonary artery (LPA) and DA were delivered by caesarean section at 126±1 days of gestation. Lambs were ventilated with a tidal volume of 5 mL/kg, 60 breaths/min, variable fraction of inspired oxygen and 5 cm H_2O positive end-expiratory pressure. LPA and DA blood flows were digitally recorded after birth. After a 30 minute stabilisation period, lambs were randomly given 40 mg/kg caffeine base i.v (n=6) or saline (control lambs; n=6), infused over 30 minutes. Lambs were then ventilated for a further 2 hours. Results: Arterial caffeine concentrations increased to 38.4 ± 3.0 mg/L ~60 minutes after starting the infusion. Heart rate also increased (212 ± 9 bpm) at this time in caffeine treated lambs compared to controls (158 ± 9 bpm). Blood flow in the LPA and DA were similar between caffeine treated and control lambs, as were all ventilation parameters and arterial blood gas status. Conclusions: Neonatal caffeine administration increased heart rate but had little effect on pulmonary function and DA blood flow in ventilated very preterm lambs. Further studies are required to elucidate the beneficial effect of caffeine in reducing CLD and rate of patent DA.

(1) Schmidt et al., Caffeine therapy for apnea of prematurity. N Engl J Med 2006; 354:2112-21

027

PERSISTENT ALTERATIONS IN THE LUNGS OF ADOLESCENT PRETERM LAMBS FOLLOWING INTRAUTERINE ENDOTOXIN EXPOSURE

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Background and aim: Intrauterine inflammation has been shown to increase surfactant production and cause an arrest in alveolarization in the ovine fetal lung. However, it remains unclear if these changes persist postnatally. Our aim was to determine the effects of intrauterine inflammation on lung structure, surfactant composition and proinflammatory cytokine expression in postnatal lambs that were born preterm.

Methods: In date-mated pregnant sheep lipopolysaccharide (LPS, 1 mg/day, n=6) or saline (n=9) was infused into the amniotic sac from 110 days gestational age (DGA) until induced preterm birth at ~133 DGA (term ~147 DGA). Necropsy was performed at 11 weeks postnatal age. Sections of the left lung were snap-frozen for molecular analysis and the right lung was processed for histological analysis. The structure of the l ung parenchyma and bronchioles were morphometrically analyzed. Relative gene expression of surfactant proteins (SP)-A, -B, -C and -D, and interleukin (IL)-1β, IL-6, IL-8 and tumor necrosis factor (TNF)-α was determined using quantitative real-time PCR.

Results: There were no differences in birth weight or postnatal growth between LPS-exposed and control lambs; however the spleen was 42% lighter and the kidneys were 13% lighter in LPS-exposed lambs. In the lung parenchyma there were no significant differences in percent tissue and airspace, alveolar size and elastin and collagen deposition. In the bronchioles there were no significant differences in epithelial area, alveolar-bronchiolar attachments and collagen and smooth muscle content in the outer airway wall. There was a significantly greater expression of lung SP-A (1.34±0.14 vs 1.00±0.03), SP-C (1.53±0.16 vs 1.00±0.11) and IL-1β (2.06±0.34 vs 1.00±0.09) in LPS-treated lambs compared to controls.

Conclusions: Intrauterine endotoxin exposure induces persistent changes within the postnatal ovine lung. Increased SP-A and IL-1β gene expression could affect the ability of the lung to clear pathogens, whilst increased SP-C gene expression may improve lung function.

028

SUPERIOR VENA CAVA BLOOD FLOW IN THE HUMAN FETUS AND THE EFFECT OF FETAL BREATHING MOVEMENTS

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Objectives: Superior vena cava (SVC) drains venous blood from the upper fetal body, mainly the head. Data is scarcely known on the human fetus. Here we present reference values for the second half of pregnancy, and test the hypothesis that fetal breathing movements (FBM) enhances this flow. Methods: Based on a power calculation, 110 women with low-risk singleton pregnancies were recruited to a longitudinal study that included three sets of observations during the second half of pregnancy. Ultrasound was used to determine inner diameter, peak systolic blood velocity and time-average maximum velocities in the SVC during rest and respiratory activity. Results: Based on 558 sets of observations obtained during fetal rest and FBM, we found VCS blood flow increased from 57.8 mL/min (95%CI 51.7-64.3) to 221.5 (204.5-239.3) during the second half of pregnancy. During FBM there was an overall increase in diameter, 0.41 cm (0.40-0.42) vs. 0.46 (0.44-0.48), peak systolic velocity, 35.9 cm/s (34.9-37.0) vs. 62.2 (59.1-65.5) and time-averaged maximum velocity, 20.3 cm/s (19.7-20.8) vs. 27.3 (26.1-28.6), respectively. This resulted in an overall 90% increase in mean SVC blood flow, from 108.1 mL/min (98.8-117.9) at rest to 205.9 (183.2.5-230.5) during FBM. Conclusion: Blood flow in the SVC increases substantially during the second half of pregnancy and is highly influenced by FBM. Since FBM additionally reduces flow in the inferior vena cava, the net effect is a prioritized venous drainage from the fetal head enhancing the washout of CO2 in that area, which also contains the chemoreceptors.

029

EFFECTS OF IBUPROFEN TREATMENT ON THE DEVELOPING PRETERM BABOON KIDNEY

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Introduction: Preterm birth is associated with the presence of morphologically abnormal glomeruli in the outer renal cortex of the developing kidney. These abnormalities are hypothesised to be caused by factors in the postnatal care of the neonate which impair renal development. Non-steroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen, are nephrotoxic drugs commonly administered to preterm neonates in order to close a patent ductus arteriosus. The combined administration of NSAIDs and nitric oxide synthase inhibitors (NOSi) has also been trialled. Aim: The aim of this study was to determine whether early postnatal non-steroidal anti-inflammatory drug treatment is the cause of the glomerular abnormalities observed in the preterm kidney. Methods: Baboon neonates were delivered prematurely at 125d gestation (term = 185d) and were euthanized at birth or at postnatal day 6. Neonates were divided into four groups: 125d gestational controls (n=4), Untreated (n=8), Ibuprofen (n=6), and Ibuprofen+NOSi (n=3). Animals in the Ibuprofen and Ibuprofen+NOSi groups received 5 doses of ibuprofen, at 24 hr intervals, with the Ibuprofen+NOSi animals additionally administered a nitric oxide synthase (NOS) inhibitor (L-NMMA). Results: There was no difference between groups in body weight, kidney weight or glomerular generation number. Nephrogenic zone width was significantly reduced in the Ibuprofen group compared to the 125d gestational control and Untreated animals. Morphologically abnormal glomeruli were present at a range of 0.0% to 22.9% in the Untreated group, 0.0% to 6.1% in the Ibuprofen group, and 0.0% to 1.4% in the Ibuprofen+NOSi group. Conclusion: Early postnatal ibuprofen exposure is associated with a reduced nephrogenic zone width; however, it is not the cause of the abnormal glomerular morphology associated with preterm birth.

030

HEART DISEASE LINK TO PRENATAL HYPOXIA AND OXIDATIVE STRESS

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INTRODUCTION: The prenatal environment interacts with our genes to determine cardiovascular risk. However, mechanisms underlying developmental programming remain elusive, precluding the identification of potential therapy. Common complications in pregnancy include reductions in oxygen and nutrient delivery to fetus. Using an integrative approach at the isolated organ, cellular and molecular levels, we show in rat pregnancy that prenatal hypoxia programmes cardiac and endothelial dysfunction in adulthood secondary to the genesis of oxidative stress in the fetal heart and circulation, and that antioxidant treatment of hypoxic pregnancies prevents this.

METHODS: This longitudinal study investigated effects of maternal treatment of hypoxic (13% O2) pregnancy with vitamin C (5 mg.ml-1 drinking water) on the cardiovascular system of the offspring at two life stages: in the fetus at the end of gestation and at 4 months of adulthood. On day 6 of pregnancy, rats (n=20 per group) were exposed to normoxia or hypoxia ± vitamin C. Maternal food intake was unaffected. At day 20, tissues were collected from 1 male fetus per litter per group from one set of dams (n=10). The remaining 10 litters per group delivered. At 4 months, tissues were either perfusion fixed, frozen, or dissected for isolated organ preparations from 1 male per litter per outcome variable.

RESULTS: In the fetus, hypoxic pregnancy promoted aortic thickening with enhanced nitrotyrosine and increased cardiac HSP70 expression. At adulthood, offspring of hypoxic pregnancy had markedly impaired NO-dependent relaxation in femoral resistance arteries, and increased myocardial contractility associated with enhanced sympathetic but depressed parasympathetic cardiac reactivity. Maternal vitamin C prevented these effects in fetal and adult offspring of hypoxic pregnancies.

CONCLUSIONS: Developmental hypoxia programmes cardiovascular disease secondary to oxidative stress. The study offers insight into mechanism and targets for intervention against a fetal origin of cardiac and peripheral vascular disease in offspring of risky pregnancy.

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031

THE EFFECT OF MELATONIN ON CARDIAC PERFORMANCE DURING ASPHYXIA IN THE PRETERM FETAL SHEEP

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Introduction: Melatonin may be given during pregnancy to reduce neural injury associated with hypoxia-ischemia. Given that it may be infused during labour, it is important to evaluate whether melatonin alters ECG parameters used to assess fetal wellbeing.

Aim: The aim of this study was to examine whether melatonin altered cardiac performance during asphyxia.

Methods: 0.7 gestation fetal sheep were instrumented to allow continuous monitoring of the fetal ECG from which fetal heart rate (FHR), long-term fetal heart rate variability (FHRV LTV) and ST segment height were derived. Fetuses received either a bolus of 0.1mg/kg followed by 0.1mg/kg/h melatonin starting 15 min before umbilical cord occlusion and continued during and for 6 hours after occlusion (n = 8) or vehicle (saline, n = 8). Asphyxia was induced by 25 minutes of complete umbilical cord occlusion.

Results: FHR fell rapidly in both groups at the onset of occlusion, but FHR was higher in the melatonin group (minutes 3-7, $p<0.05$). ST height increased in both groups, peaking at minute 6, and returning to baseline by 9 minutes. However, the rise in ST height was significantly less in the melatonin group (183.6±20 vs. 302.8±52 at 6 min, $p<0.01$) There was an increase in FHRV LTV in both groups during the first 7 minutes of occlusion, peaking at minute 2, with no difference between groups. There was a secondary rise in FHRV LTV in the control group from 20 minutes of occlusion, but no rise in the melatonin group. Analysis of the ECG showed that the secondary rise in FHRV LTV was associated with periods of irregular heart rhythms such as bigeminy and tachycardia followed by further bradycardia.

Conclusion: Our study suggests that fetal exposure to melatonin prior to and during asphyxia appears to be associated with better cardiac performance which may play a role in reducing brain injury and may act to protect the heart from injury.

032

INCREASED PHOSPHORYLATED CAMKII MAY BE RESPONSIBLE FOR CARDIAC HYPERTROPHY IN LBW LAMBS

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Rationale: IGF-2R is known to function as a clearance molecule to reduce IGF-2 bioavailability. However, recent *in vitro* rat studies indicate that IGF-2R has the ability to induce cardiomyocyte hypertrophy in a G protein coupled receptor (Gαq)-dependent manner in the heart. It is not known, however, if slow growth before birth activates the IGF-2R signalling pathway in postnatal life.

Objective: To determine if the increased IGF-2R gene expression in the low birth weight (LBW) lambs activates the downstream molecules of the Gαq signalling pathway to cause cardiac hypertrophy in LBW lambs.

Methods: Carunclectomy was performed 10 weeks prior to mating to induce LBW. Hearts were collected from average birth weight (ABW) and LBW lambs at 21d of age. We used qRT-PCR to quantify cardiac mRNA expression of IGF-2 and IGF-2R normalised to the housekeeper, RpP0. We used Western blotting to quantify cardiac protein expression of Ca2+/calmodulin-dependent protein kinase II (CaMKII) and phosphorylated CaMKII.

Results: At 21d, LBW lambs had a smaller body weight and left ventricle (LV) weight compared to ABW lamb. LBW lambs, however, had a greater LV weight relative to body weight. Cardiac IGF-2 and IGF-2R mRNA expression were significantly higher in LBW compared ABW lambs. LV weight relative to heart weight was significantly related to cardiac IGF-2 mRNA expression ($y=0.578x+2.467$; $r2=0.37$; $P<0.05$). There was no change in cardiac CaMKII protein expression between the groups, but a higher phosphorylated CaMKII protein expression in LBW lamb compared to ABW lamb. Heart weight was negatively related to phosphorylated CaMKII protein ($y=-0.003x+93.271$; $r2=0.325$; $P<0.05$).

Conclusions: In LBW lambs, IGF-2 stimulates IGF-2R signalling pathway which results in increased phosphorylated CaMKII leading to cardiac hypertrophy.

033

CORONARY AND AORTIC FLOW IN RESPONSE TO CHANGES IN PRELOAD AND AFTERLOAD IN THE ISOLATED PRETERM PIGLET HEART

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Background: We have previously shown in the isolated working heart that preterm piglets have reduced cardiac output (per kg BW) compared to term piglets and are unable to maintain systemic (aortic) flow at high afterloads.

Aims/Hypothesis: To assess in the isolated preterm piglet heart a) the effects of afterload on coronary flow and, b) the effects of maternal glucocorticoid treatment on the ability of the preterm heart to produce adequate systemic blood flow.

Methods: Piglets were delivered by caesarean section at term (115d) or preterm (92d) and an additional group of preterm piglets received maternal glucocorticoid treatment 48h and 24h prior to delivery. An isolated working heart model was used to assess cardiac output (mL/min/kg BW), aortic flow (mL/min/kg BW), coronary flow (mL/min/g heart), contractility (d*P*/dtmax) and developed pressure (mmHg).

Results: Contractility and developed pressure were similar in term and preterm hearts. In preterm hearts elevations in afterload markedly depressed aortic flow, with cardiac output disproportionately redistributed to coronary vessels at high afterloads. As a result, >55% of preterm hearts were unable to maintain adequate aortic flow at afterloads ≥30mmHg. The cardiac work:coronary flow relationship was similar in glucocorticoid exposed preterm hearts and term hearts but suggested a greater requirement for coronary flow as afterload increased in preterm pigs. Exposure to maternal glucocorticoids increased the proportion of preterm hearts able to maintain adequate flow at low afterloads.

Conclusions: T he preterm heart may lack the functional capacity to acutely adapt to post-natal afterload as a result of a disproportionate increase in coronary flow. To maximise aortic flow and specifically SBF in preterm infants, treatments limiting afterload while harnessing significant preload reserve should be targeted. Exposure of preterm hearts to maternal glucocorticoids improved systemic blood flow at low to moderate afterloads through either increased of cardiac efficiency or improved coronary vascular tone.

034

ACUTE INFLAMMATION *IN UTERO* ADVERSELY ALTERS CEREBRAL BLOOD FLOW (CBF) AND BRAIN INJURY IN PRETERM LAMBS.

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Background: Brain injury is common in extremely preterm infants, particularly in those requiring respiratory support. The initiation of ventilation is a high risk period for postnatal brain injury. Inflammation *in utero* increases the risk and severity of brain injury. We investigated the effect of ventilation on brain inflammation and injury, and determined whether inflammation *in utero* exacerbates ventilation associated brain injury.

Design/Methods: Preterm lambs were exposed to either intra-amniotic lipopolysaccharide (LPS; 10 mg) or saline (2 mL) 2 d prior to surgical implantation of ultrasonic flow probes and catheters for assessment of pulmonary and cerebral haemodynamics. Lambs were delivered at 128 ± 1 d and placed on mechanical ventilation (Dräger) with a positive end-expiratory pressure of 4 cmH2O; peak inspiratory pressure was adjusted to target a tidal volume of 7 mL/kg. Animals were monitored with continuous oximetry and frequent blood gas sampling. At autopsy tissue was collected for molecular and histological assessment of lung and brain injury and compared to unventilated controls (UVC).

Results: Oxygenation and ventilation parameters were similar between groups, but CBF was higher and more variable in 2d LPS lambs compared to controls. LPS UVC had higher brain interleukin (IL)-8 mRNA compared to saline UVC, but ventilation after LPS significantly increased brain IL-1β, IL-6 and IL-8 mRNA compared to all other groups. Lung proinflammatory cytokine mRNA expression was not different between groups. The incidence and severity of infiltrating inflammatory cells, gross anatomical injury, vascular leakage and haemorrhage in the deep white matter of the brain were increased by ventilation, and further exacerbated by LPS *in utero*.

Conclusion: Ventilation after acute inflammation *in utero* increases inflammation in the deep white matter of the brain and increases the incidence and severity of gross anatomical injury and haemorrhage in the brain.

035

ASTHMA DURING PREGNANCY INCREASES THE RISK OF SIGNIFICANT NEONATAL MORBIDITY

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Background: Asthma is the most prevalent chronic condition to affect pregnancies in Australia, currently affecting 12% of pregnant women and expected to rise to 20% in the next five years. We have previously reported an association between maternal asthma and adverse perinatal outcomes, including preterm delivery, still birth and intrauterine growth restriction (IUGR), and other studies suggest an increased risk for congenital malformations. This study aimed to examine the effect of asthma during pregnancy on perinatal birth outcomes and congenital malformation rates in a South Australian cohort.

Method: All singleton birth outcomes in South Australia over ten years (1999-2008; n=178,000) were analysed to assess the effect of asthma on perinatal outcomes. Logistic regression was used to calculate odds ratios and adjust for factors including smoking, maternal age and degree of prematurity.

Results : Asthma was reported in 6.5% of pregnancies, and was associated with a 27% increased risk of preterm delivery (95% CI 1.19-1.36). This effect remained after adjusting for maternal smoking, parity, maternal age and gestational diabetes (OR=1.21, 95%CI 1.13-1.30). Congenital abnormalities were more frequent in pregnancies associated with asthma (3.9% versus 2.4%; $p<0.001$). An increased requirement for resuscitation (OR=1.15, 95%CI 1.08-1.23) and oxygen therapy >4hours (OR=1.12, 1.03-1.22) was also observed in pregnancies associated with asthma after adjusting for preterm birth, explaining the significant increase in neonatal intensive care admission rates (2.8% versus 2.2%; $p<0.001$).

Conclusion: An increased risk of preterm delivery and congenital malformations were observed in pregnancies complicated by asthma. Importantly, resuscitation and oxygen therapy were required by neonates of mothers with asthma irrespective of the degree of prematurity. This study has therefore highlighted maternal asthma as a significant contributor to neonatal morbidity.

036

CHILDREN BORN PRETERM HAVE REDUCED LONG TERM DEPRESSION (LTD)-LIKE NEUROPLASTICITY

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Background: Neuroplasticity is the ability of the brain to alter neuronal synaptic strength in response to activity and experiences, is widely accepted to be the mechanism underlying learning and memory formation. Preterm children have alterations in cortical development, functional connectivity and neural activation pattern that suggests their capacity for neuroplastic reorganization may also be reduced, critically contributing to their common difficulties with learning and memory.

Hypothesis: Preterm birth is associated with a reduced response to a non-invasive neuroplasticity induction intervention designed to induce a short-term LTD-like (i.e. inhibitory) change in motor cortex (M1) excitability.

Methods: 25 children (15 females) aged 12-15 years (13.67 ± 0.48 years) participated; Term born (37-41 wks GA) N=6, Late preterm (33-36 wks GA) N=9 and Early preterm (24-32 wks GA) N=9. Continuous theta burst stimulation (cTBS) was applied to the M1 to induce LTD-like neuroplasticity. To assess changes in M1 excitability (an indicator of neuroplasticity), transcranial magnetic brain stimulation was used to evoke motor evoked potentials (MEPs) from a hand muscle before and up to 60 min following cTBS.

Results: Term-born children showed robust motor cortex inhibition immediately following cTBS that was greater and more persistent than that previously consistently recorded in adults. In comparison, inhibition in both preterm groups was significantly less than term born children and returned to baseline within 40 min of cTBS ceasing. GA correlated negatively with the mean MEP inhibition following cTBS, i.e. the least inhibition was evoked in the most preterm children.

Conclusions: These data provide the first physiological evidence of reduced neuroplasticity in preterm children. While different types of neuroplasticity induction (i.e. LTP-like, behavioural) are yet to be assessed, these results demonstrate that even modest levels of prematurity are associated with significant impairments that persist at least into early adolescence. The underlying mechanisms are not yet clear, but may include synapse specific dysfunction and/or altered cortisol secretion patterns which are known to influence neuroplasticity.

037

MECHANISM OF REDUCTION OF UMBILICAL ARTERIAL METABOLIC ACIDEMIA FOLLOWING APPLICATION OF A STANDARDIZED RULE-BASED FHR MANAGEMENT SCHEMA

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Background and Aim: We have reported a 7-fold reduction in newborn metabolic acidemia (BE<-12mEq/l) after intensive training of providers and hospital wide adoption of a rule-based 5-category FHR management framework, without a change in operative delivery rates. We sought evidence for the relationship being causal by detailed analysis of FHR characteristics and acid-base and blood gas data before and after training.

Materials and Methods: Rates of umbilical arterial pH and base excess were determined over a 5 year period in a single hospital with 3907 deliveries in Japan. We compared results in the 2 years before and after a 6 month training period in the FHR management system.

Results: After the training period there was an increase in the percentage of normal patterns, and a decrease in variable decelerations, late decelerations and prolonged decelerations in the last 60 minutes of labor,. Variable decelerations decreased on average by 20%, late decelerations by 50%, and prolonged decelerations by 30%. There was also a reduction in umbilical arterial metabolic acidemia (base excess) in the various deceleration groups after introduction of the FHR management framework.

Conclusions: The adoption of this FHR management system resulted in a reduction of decelerations and metabolic acidemia, without a change in cesarean or vacuum delivery rates. These results indicate that the obstetrical providers were able to better select for intervention those patients destined to develop acidemia, demonstrating a causal relationship between the management system and reduced decelerations and metabolic acidemia.

038

WHY IS SLEEPING IN THE PRONE POSITION A RISK FOR THE SUDDEN INFANT DEATH SYNDROME?

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Introduction: Despite the dramatic reduction in the Sudden Infant Death Syndrome (SIDS) by over 80% since the introduction of safe sleeping campaigns, SIDS still remains the leading cause of postnatal death in western countries. SIDS has a unique age distribution with over 90% of infants dying in the first 6 months of life, with a distinct peak at 2-3 months of age. Currently it is believed that SIDS occurs during sleep, and that a failure of cardiorespiratory control, together with a failure to arouse from sleep, is involved in the final pathway. Over the last 15 years our group has been investigating how the major risk factor for SIDS (prone sleep) alters physiology during sleep in healthy infants to try and better understand the mechanisms of SIDS.

Methods: Studies in infants have been carried out longitudinally at 2-4 weeks, 2-3 months and 5-6 months of age using daytime polysomnography to investigate the effects of prone sleeping on cardiovascular control and arousability.

Results: When infants sleep prone arousability from sleep is depressed 3 fold. Furthermore, there is a fall in blood pressure despite an increase in heart rate, and cardiovascular control is impaired and this is most marked at 2-3 months of age. Recently, we have shown that prone sleeping is also associated with reduced cerebral oxygenation.

Conclusions: In normal healthy infants cerebral oxygenation decreased with postnatal age and this was most marked between 2-4 weeks and 2-3 months of age. This reduction may underpin the decreased arousability from sleep exhibited by normal infants in the prone position. Studies in healthy infants can provide important insights into the likely mechanisms for SIDS and provide important evidence for SIDS prevention campaigns.

039

PODOCYTES ARE FOUND IN THE URINE OF PRETERM INFANTS RECEIVING INDOMETHACIN TREATMENT SUGGESTING DRUG INDUCED GLOMERULAR INJURY.

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Introduction: Preterm infants are delivered while glomerulogenesis is ongoing and thus may be exposed to a number of insults that may affect renal development. Podocytes detected in the urine are an indicator of glomerular injury in a number of renal conditions. The aim of this study was to determine whether preterm and term infants excrete podocytes in their urine and if exposure to gentamicin and indomethacin increased podocyte excretion in their urine.

Methods: Preterm infants less than 33 weeks gestation had urine collected each day while receiving either gentamicin or indomethacin and the number of casts and podocytes present in the urine were compared with preterm and term control infants urine who did not receive gentamicin or indomethacin.

Results: Forty two neonates were included in the study. Podocytes were present in small numbers (< 2) in the urine of both preterm and term control neonates. There were increased numbers of podocytes in the urine of preterm neonates receiving indomethacin (p=0.02).

Conclusions: The increased number of podocytes in preterm neonates receiving indomethacin suggests that glomerular injury is occurring. It is unknown whether injury to glomeruli during glomerulogenesis in preterm neonates has long-term sequelae for renal development and function into adulthood.

040

SEX AND PROGESTERONE: IMPROVING PRETERM SURVIVAL.

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Preterm birth and intrauterine growth restriction are major risk factors for perinatal brain injury. The neurosteroid allopregnanolone, a metabolite of progesterone via 5α-reductase (5αR) action, modulates neural excitability, has neuroprotective properties and has been shown to influence respiratory function. Concentrations of progesterone and allopregnanolone are high during gestation but fall markedly after birth. We aimed to establish an animal model for the study of neuroactive steroid replacement in preterm neonates to improve survival and neonatal outcomes.

Guinea pig neonates were delivered by c-section at 62-63 days gestation (preterm) or at 69 days (term=70 day) following betamethasone treatment 24hrs prior to delivery. Neonates received CPAP, surfactant, assisted feeding and were housed in a humidified incubator. In order to assess the potential of progesterone therapy preterm neonates received progesterone (16mg/kg) or vehicle (2-hydroxypropyl β-cyclodextrin) injections at 1 and 6hrs. Physical characteristics were recorded for each animal. Brain to liver weight ratios (BLR) were calculated as a measure of assymmetric growth and brain sparing. Animals were euthanased at 24hrs and brains and other organs collected for analysis. Preterm neonates were categorised by survival to 24hrs as survivors (24hrs) and non-survivors (<24hrs).

Animals that died within 1hr of birth had significantly reduced birth weights compared to those of animals that survived past 1hr. Progesterone treated animals at 24hrs had significantly increased neonatal plasma and brain allopregnanolone concentrations when compared to vehicle controls. Progesterone treatment increased male survival at 24hrs from 38% to 62%. Progesterone treated males had significantly lower birth weights than vehicle treated males at 24hrs, whilst there was no difference in birth weights in non-surviving neonates.

These data may support a role for progesterone in improving outcomes in preterm male neonates. The effect of progesterone and neurosteroids on the developing preterm brain as potential therapeutic agents warrants further investigation, particularly in light of these sex-specific actions.

041

PRENATAL LOW DOSE ETHANOL EXPOSURE ALTERS GLUCOSE HOMEOSTASIS IN RATS.

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Background: Exposure to a sub-optimal uterine environment may interfere with fetal growth, physiology and metabolism and has been linked with metabolic diseases in adulthood. Previous studies have shown that *high* prenatal ethanol exposure (PEE) is associated with altered glucose homeostasis in rat offspring. It is unknown whether *low* PEE produces similar outcomes. The aim of this study was to determine if *low* PEE impairs glucose homeostasis.

Methods : Male and female Sprague Dawley rats prenatally exposed to 6% (v/v) ethanol were utilised for this study. Fasting plasma glucose and insulin concentrations were measured at 2 and 4 months of age. At 4 months, intraperitoneal glucose (1g/kg) and insulin (1U/kg) tolerance tests (IPGTT and IPITT) were performed to determine glucose tolerance, insulin secretion and insulin sensitivity.

Results : PEE did not alter offspring body weight between postnatal day 1 and 4 months of age. At 2 months fasting glucose and insulin levels were not different between groups. At 4 months, fasting glucose concentrations were also similar between groups, with no alterations in plasma glucose during the IPGTT or IPITT. However, at this age basal insulin was elevated in PEE animals ($P<0.05$ in males and $P=0.06$ in females compared to control) and PEE offspring reached a significantly higher peak plasma insulin at 5mins.

Conclusions : PEE results in normal early postnatal growth and normal basal plasma glucose concentrations up to 4 months of age. However, at 4 months these offspring require higher insulin concentrations for normal glucose homeostasis and display subtle changes in glucose handling, suggesting insulin resistance at 4 months.

042

EFFECTS OF VOLUNTARY EXERCISE ON ETHANOL NEUROBEHAVIOURAL TERATOGENICITY IN THE GUINEA PIG.

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Introduction: Ethanol consumption during pregnancy can produce a spectrum of teratogenic effects in offspring, termed fetal alcohol spectrum disorders. The most debilitating consequence is neurobehavioural teratogenicity. Voluntary exercise (VE) can mitigate brain injury by various mechanisms, including increased neurogenesis. The objective of this study was to determine the effects of short-term structured VE on ethanol neurobehavioural teratogenicity.

Methods: Pregnant Dunkin-Hartley-strain guinea pigs received ethanol (4 g/kg maternal body weight/day, 5 days/week throughout gestation) or isocaloric-sucrose/pair-feeding (nutritional control). Spontaneous locomotor activity (SLA) was measured in offspring at postnatal day (PD) 10. Offspring of each treatment group were randomly assigned to one of two intervention groups: VE or no intervention (NI). VE animals were placed in a dry-land maze in same-sex pairs for 30 min daily for 21 days (PD 24-44). Performance of each ethanol-exposed (EE) or control offspring in the maze was measured. Following VE or NI, SLA was measured in the offspring at PD 45.

Results: EE offspring, compared with control, had decreased birthweight and brain weight at PD 150-200, and increased SLA (increased distance traveled, time moving and time hyperactive) at PD 10 ($p < 0.05$). During VE, EE and control offspring were not different for % completed trials or time to complete trials, whereas EE animals made more errors (wrong turns and entering dead ends)($p < 0.05$). EE offspring demonstrated less SLA at PD 45 compared with PD 10 for each of NI and VE ($p < 0.05$). Control offspring demonstrated similar SLA at PD 45 and 10 for NI, and less SLA at PD 45 compared with PD 10 for VE ($p < 0.05$).

Conclusions: There is an age-dependent decrease in the magnitude of EE-induced increased SLA, and VE decreases SLA in EE and nutritional-control offspring. Supported by CIHR MOP 81185 and ELA 80227.

043

DIET INDUCED PATERNAL OBESITY IMPAIRS THE METABOLIC AND REPRODUCTIVE HEALTH OF TWO SUBSEQUENT GENERATIONS.

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Paternal exposure to a high fat diet and resultant obesity and diabetes in rodents, impairs glucose tolerance, due to insulin deficiency, in female offspring,1 identifying a novel pathway for intergenerational transmission of metabolic disease. Paternal diet induced male obesity in mice, in the absence of hyperglycemia, affects sperm motility and increases sperm DNA damage, impairing embryo development and reducing pregnancy rates.2, 3 We therefore examined the effects of diet induced obesity in male mice, in the absence of diabetes, on health of offspring across two generations. Male C57BL6 mice (n=16 per diet) were fed a high fat diet (HFD, 21% fat) from 5 weeks of age for 12 weeks, increasing body weight and adiposity, but not altering glycaemia, when compared to males fed a control diet (CD, 6% fat). Males were then mated to control females, and metabolic and reproductive health of the offspring (F1) (n=32/sex/diet) assessed to 9 months of age. FI offspring were then mated to control fed mice and health of their offspring (F2) was similarly assessed. Paternal high fat diet exposure and obesity reduced the number of litters produced, consistent with our previous findings of male sub-fertility. 2, 3 Paternal diet induced obesity also induced insulin resistance and obesity in offspring, with earlier and more marked onset in females. Furthermore, gametes from both male and female offspring of obese fathers had impaired reproductive capacity and evidence of oxidative stress. In addition, there was intergenerational transmission of impaired reproductive function through both parental lines to the second generation and of insulin resistance through the paternal line to females and through the maternal line to males.This is the first demonstration of high fat diet induced paternal intergenerational transmission of obesity, insulin resistance and impaired reproductive function and of their further intergenerational transmission through both parental lines.

(1) Ng et al 2010 Nature 467: 963
(2) Bakos et al 2011 Fertil Steril 95: 1700
(3) Mitchell M et al 2011 Fertil Steril 95: 1349

044

STATINS PREVENT ADVERSE EFFECTS OF POSTNATAL DEXAMETHASONE THERAPY ON CARDIOVASCULAR FUNCTION IN WEANLING RATS

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INTRODUCTION: Dexamethasone (Dex) therapy in premature infants reduces chronic lung disease (CLD) but it also triggers cardiac (1) and vascular (2) dysfunction. The mechanisms underlying these detrimental effects of Dex are unclear, but oxidative stress with subsequent depletion of nitric oxide (NO) may be involved (3). In addition to lowering cholesterol, statins increase NO (4). Using an integrative approach *in vivo* and the levels of the isolated heart and vasculature, this study tested the hypothesis that combined treatment with statin prevents Dex-induced cardiovascular dysfunction.

METHODS: Male Wistar rat pups (52 litters) received a 3-day tapering course of Dex (0.5, 0.3 and 0.1 m g.g-1.day-1 i.p.) or saline (10 m l.g-1.day-1 i.p.) without or with pravastatin (10mg.kg-1 i.p.). Pravastatin or vehicle continued from P4-6. One male from each litter was used for anyone variable outcome. At P21, *in vivo,* under urethane anaesthesia, cardiac baroreflex responses were generated (phenylephrine, 5-80 m g.kg-1 i.v.). Following euthanasia, cardiac function was investigated in a Langendorff preparation. Femoral artery reactivity was assessed by wire myography (phenylephrine 10-9M-10-5M and metacholine 10-9M-10-4M).

RESULTS: Relative to controls, Dex treatment increased mean arterial blood pressure (67.0±2.1 *vs*. 60.0±2.1 mmHg, n=7, $P<0.05$) and baroreflex gain (2.1±0.2 *vs*. 1.3±0.1 bpm.mmHg-1, n=7, $P<0.05$). *In vitro,* Dex reduced left ventricular developed pressure (LVDP, 40.5±2.5 *vs*. 70.9±5.4 mmHg, n=10, $P<0.05$) and myocardial contractility (dP/dtmax, 3208.7±250.4 *vs.* 4931.9±324.1 mmHg.s-1, n=10, $P<0.05$) and delayed cardiac recovery to 15 min of ischaemia. Wire myography revealed that Dex increased reactivity to phenylephrine but it decreased endothelial dependent relaxation to metacholine. Concomitant treatment with pravastatin restored all cardiovascular dysfunction triggered by postnatal Dex. Pravastatin alone had no effect on cardiovascular function.

CONCLUSIONS: Statins protect against hypertension, cardiac and endothelial dysfunction following postnatal glucocorticoid therapy. Combined glucocorticoid and statin therapy may be safer than glucocorticoid alone in the treatment of CLD in premature infants.

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(1)Bal et al. Ped Res, 58(1):46,2005
(2) Herrera et al. PLoS One, 5(2):e9250, 2010
(3) Iuchi et al.Circ Res, 92(1): 81,2003
(4) Kaesemeyer et al. JACC, 33(1):234, 1999

045

DEVELOPMENTAL PROGRAMMING OF NEUROLOGICAL DISEASE: ROLE OF ADVERSE PRE- AND NEO-NATAL CONDITIONS AND OXIDATIVE STRESS

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Three major challenges to the developing fetus; increased glucocorticoids (1,2), undernutrition (3) and hypoxia (4), can programme disease in adulthood, secondary to excessive ROS generation. This raises the possibility that oxidative stress could be a common pathway via which challenges to the fetus programme disease in adult life. We have explored this concept in relation to cerebral structure and function, in two experimental models which involve pre- or neo-natal challenges.

Wistar dams underwent pregnancy under normoxic (21% O2) or hypoxic (13% O2) conditions from days 6-21, with or without vitamin C (0.5g.100ml-1 in drinking water). In offspring at 3.5 months of age, Morris water maze and open field testing was performed to assess cognitive function and exploratory behaviour. In a separate study, male Wistar pups received either DEX (0.5, 0.3, 0.1 g.g-1, i.p.), DEX with vitamins C and E (200 mg.kg-1 and 100 mg.kg-1, respectively), pravastatin (10 mg.kg-1), saline, or saline with antioxidants from postnatal days 1-3 (P1-3). Antioxidants were continued from P4-6. At P21, the cortex and hippocampus were isolated for Western blot analysis; brain tissue was collected at P22 for histological analysis.

Prenatal hypoxia impaired memory retention in adult offspring, an effect which was diminished in offspring of hypoxic pregnancy treated with vitamin C ($P<0.05$). Neo-natal treatment with DEX increased three indices of oxidative stress in the cortex (Hsp70, 4-HNE and NT), decreased soma volume of neurons in the CA1 and dentate gyrus of the hippocampus ($P<0.05$). Co-administration of vitamins C and E or statins in DEX-treated pups restored Hsp70 protein expression, and soma volume of neurons in the CA1 and dentate gyrus ($P<0.05$).

Alterations in brain structure and function induced by common pre- or neo-natal challenges can be ameliorated by antioxidant treatment, suggesting that oxidative stress may be a key link in the developmental programming of neurological disease.

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

(1)Iuchi et al. (2003) Circ Res 92: 81-87.
(2) Wallwork et al. (2003) Microvasc Res 66: 30-37.
(3) Franco Mdo et al. (2003) Cardiovasc Res 59: 767-775.
(4) Niu et al. (2010). Presented at Society for Gynecologic Investigation, Orlando, U.S.A. Reproductive Sciences abstract 342.

046

PERICONCEPTIONAL UNDERNUTRITION ALTERS INSULIN SIGNALLING IN MUSCLE FROM LATE GESTATION SHEEP FETUS

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Introduction: Maternal undernutrition during early gestation in human is associated with an increase risk of insulin resistance and glucose intolerance in adulthood. This study aims to investigate the effect of maternal undernutrition during the periconceptional and early preimplantational period (PCUN and PIUN) on factors regulating insulin signalling in late gestational fetal muscle.

Hypothesis: We hypothesised that PCUN and PIUN would result in a decrease in gene expression of the insulin receptors A and B (IRA,IRB) and a decrease in the protein abundance of the insulin signalling factors.

Methods: Control ewes were fed 100% metabolisable energy requirement (MER) from -45d to 6d postconception. Ewes in the PCUN group were fed 70% MER from -45d to 6d and ewes in the PIUN group were fed 70% MER from conception to 6d postconception. Skeletal muscle samples from singleton fetuses were collected at 136-138d gestation. The mRNA expression of the insulin receptors IRA and IRB were analysed using Real Time-PCR. The protein abundance of the insulin signalling factors IR, IRS1, p85, p110β, Akt1, Akt2, pAkt, PKCζ and GLUT-4 were analyse using western blot.

Result: IRA and IRB mRNA expression were lower in the PCUN compared to control group (P=0.005, P=0.009, respectively), however, there was no difference in the IR abundance between the treatment groups. There were no significant difference in the abundance of IRS1, p85, Akt1, Akt2, pAkt and GLUT-4. The abundance of p110β, however, tended to be lower in the PCUN group only (P=0.065), while, the abundance of PKCζ was lower both in the PCUN and PIUN (P=0.034).

Conclusion: Findings of this study suggest that there are specific factors which are recruited after exposure to undernutrition during the periconceptional and early preimplantational periods which alter gene and protein expression in the insulin signalling pathway in skeletal muscle before birth.

047

PRENATAL CHRONIC HYPOXIA AFFECTS INSULIN SIGNALLING IN ADULT MALE RAT OFFSPRING

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Numerous epidemiological studies have shown that adverse intrauterine conditions such as undernutrition can programme metabolic and cardiovascular diseases in the offspring in later life (1, 2) . There is some evidence showing that prenatal hypoxia can lead to early origins of cardiovascular disease (3,4), however very few studies have investigated whether prenatal hypoxia programmes metabolic diseases. This study used a rat model to investigate the effects of chronic hypoxia during pregnancy on insulin signalling protein expression in 3 month old adult offspring. We also investigated whether maternal antioxidant treatment protected against alterations in insulin signalling protein expression resulting from maternal hypoxia.

METHODS: On day 6 of pregnancy rats were randomised to four groups: normoxic, normoxic with vitamin C (0.5 g.100ml-1 in drinking water), hypoxic (13% O2) and hypoxic with vitamin C (n=6 per group). This experimental model of hypoxia did not affect maternal food intake. At birth litters were culled to 8 pups. Following weaning, pups were maintained until adulthood. At 3 months, 1 male per litter was subjected to a glucose tolerance test (GTT). Following euthanasia, skeletal muscle from the same male were snap frozen for analysis of insulin signalling proteins by Western blotting.

RESULTS: At 3 months, offspring of hypoxic pregnancy relative to controls had lower serum insulin but similar blood glucose levels during the GTT. Skeletal muscle from offspring of hypoxic pregnancy showed an increase in insulin receptor β (IRβ), insulin receptor substrate 1 (IRS-1) and glucose transporter 4 (GLUT4). These data are indicative of increased insulin sensitivity in young offspring of hypoxic pregnancy. Maternal supplementation with vitamin C during hypoxic pregnancy prevented these changes from occurring.

CONCLUSION: Chronic prenatal hypoxia and oxidative stress alter the expression of insulin signalling molecules in adult offspring and these conditions could have a role in programming metabolic disease in later life.

(1)Barker DJ, Martyn CN: The maternal and fetal origins of cardiovascular disease. J Epidemiol Community Health 46:8-11, 1992.
(2) Phillips DI, Hirst S, Clark PM, Hales CN, Osmond C: Fetal growth and insulin secretion in adult life. Diabetologia 37:592-596, 1994.
(3) Rouwet EV et al: Hypoxia induces aortic hypertrophic growth, left ventricular dysfunction, and sympathetic hyperinnervation of peripheral arteries in the chick embryo. Circulation 105:2791-2796, 2002

(4) Morton JS, Rueda-Clausen CF, Davidge ST: Flow-mediated vasodilation is impaired in adult rat offspring exposed to prenatal hypoxia. J Appl Physiol. (In press), 2011.
(5) Fernandez-Twinn DS, Ozanne SE: Mechanisms by which poor early growth programs type-2 diabetes, obesity and the metabolic syndrome. Physiol Behav 88:234-243, 2006.

048

CHANGES IN THE INTRAUTERINE ENVIRONMENT DUE TO MATERNAL POLY I:C ADMINISTRATION IN THE SPINY MOUSE

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Background: There is considerable human and animal based evidence to support an association between maternal illness during pregnancy and adverse effects on the long-term health of the offspring. The development of mental health conditions such as attention deficit disorder, autism, and schizophrenia have been linked to changes in maternal health and the intrauterine environment that arise from viral infections. Poly I:C is a viral mimetic that targets the TLR3, a receptor of the innate immune system although the downstream effects in pregnancy is not well established. *Aims:* The aim of this study was to determine the acute immune response to a viral mimetic during pregnancy in the mother, fetus and placenta. *Methods:* Pregnant spiny mice were injected with 0.5, 1, or 5 mg/kg Poly I:C or phospahe-buffered saline (n=4/treatment) at 20 days gestation (term is 39 days). Dams were killed at 1 and 24 hours post-injection for plasma and tissue collection. Maternal brains, spleen, plasma, uterus and placental and fetal tissues were collected for qPCR analysis of TLR3, NF-κB, IFN-γ, IL-6 and TNF-α mRNA expression. *Results:* A reduction in TLR3 mRNA expression was found in the maternal spleen, placenta and fetal brain at both 1 and 24 hours after Poly I:C administration. A reduction was also found in mRNA expression of NF-κB in the maternal brain, maternal spleen and placenta at 1hr and 24hr after Poly I:C administration. In addition, a reduction in IL-6 and TNF-α mRNA expression was found in the placenta and fetal brain at 1 and 24hrs after Poly I:C adminstration. *Conclusion:*: Our model of viral infection in the pregnant spiny mouse has shown changes in the innate immune response of the fetus, placenta and mother. This study provides evidence of some of the short term consequences of a viral infection during pregnancy that are likely to contribute to the subsequent postnatal neurodevelopmental affects seen in these offspring.

049

LACK OF FLUOXETINE EFFECTS IN POSTNATAL LAMBS

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BACKGROUND: Poor neonatal adaptation, comprising transient respiratory difficulty, jitteriness and feeding difficulties is increased in human newborns exposed *in utero* to selective serotonin reuptake inhibitors (SSRIs). Several mechanisms underlying these symptoms have been proposed including acute toxicity to the drugs (i.e. serotonin syndrome), a drug withdrawal syndrome or SSRI -elicited altered fetal brain development. In this study, we identified the physiological and behavioral changes in newborn lambs (~ postnatal day 2) with acute FX administration.

METHODS : On day 3.2 ± 0.7 or 3.6 ±0.5 sterile water (0.1ml/kg, N=5) or FX (1mg/kg, N=8) was acutely injected i.v.. Arterial pressure, heart rate, ECG, arterial blood gases, pH, glucose and lactate were measured at -15, 5, 15, 30, 60 and 120 minutes. Sleep-activity cycles were measured using An Actiwatch around the neck of the lambs and this analysis was also done on another normal term lamb group (n =7), not subject to surgery or experimentation. Lamb behavior was also assessed by digital video recordings. 1 way ANOVA test was used to compare the differences in activity score. Other variables were analyzed by the 2 way ANOVA test with Bonferroni's correction. $P< 0.05$ defined significant.

RESULTS: The FX dose achieved plasma concentrations similar to those found in 2 day old human newborns exposed in utero to the drug. No significant differences were found in any of the measured variables between the experimental and control groups.

CONCLUSIONS: There is a lack of FX effects on cardiovascular-respiratory and behavioral functions in 3.6–day old postnatal lambs with acute FX administration. Thus SSRI toxicity is unlikely to be the mechanism underlying poor neonatal adaptation in human infants exposed to the drugs *in utero*.

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050

PRENATAL ALCOHOL EXPOSURE CAUSES PERSISTENT CHANGES IN VASCULAR FUNCTION AND PASSIVE MECHANICAL ARTERIAL WALL PROPERTIES IN THE OFFSPRING

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Introduction

Prenatal alcohol exposure in late pregnancy in sheep causes widespread stiffening of artery walls and changes in endothelial vasodilator function in the late gestation fetus. Our present aim was to investigate if these changes in vascular stiffness and function persist into postnatal life.

Methods

From d95-d135 of gestation (term ~145d), ethanol (0.75g/kg) was infused iv daily into ewes (n=7) over 1hr, and control ewes (n=7) received saline. The lambs were born naturally and at 8 weeks after birth, pulse wave velocity (PWV) and baroreceptor sensitivity were measured in vivo. At 9 weeks lambs were euthanized and small coronary, mesenteric, renal, femoral and cerebral arteries were isolated for testing of reactivity and wall stiffness using wire and pressure myographs.

Results

Maternal and fetal plasma ethanol concentrations peaked at ~0.11g/dL at 1hr after infusion onset and declined to zero by 8hrs. Endothelium-dependent relaxation was impaired in renal and femoral arteries of ethanol-exposed lambs ($P<0.001$). This was attributed to reduced contributions of vasodilator prostanoids and endothelium-derived hyperpolarizing factor in renal arteries and reduced nitric oxide in femoral arteries. Smooth muscle relaxation evoked by the nitric oxide donor sodium nitroprusside was impaired in renal and femoral arteries, while smooth muscle contraction was unaltered in any of the arteries. Arterial wall stiffness was altered in most vascular beds of ethanol-exposed lambs. Some arterial beds exhibited increased stiffness while others were more compliant, and these changes were sex-dependent. PWV and baroreceptor sensitivity were reduced in ethanol-exposed lambs ($P=0.006$ and 0.003, respectively).

Conclusions

Endothelial vasodilator dysfunction in ethanol-treated fetuses persists after birth in some vascular beds. The widespread arterial stiffening observed in ethanol-treated fetuses is altered postnatally, with some vascular beds having increased compliance, and these changes are associated with lower PWV. The persistent changes in postnatal vascular function and baroreceptor sensitivity induced by prenatal ethanol may increase the risk of cardiovascular disease in adulthood.

051

MATERNAL NUTRIENT RESTRICTION ALTERS EXPRESSION OF GENES THAT REGULATE BABOON FETAL PREADIPOCYTE MATURATION

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Suboptimal intrauterine nutrition predisposes to central obesity and metabolic syndrome in adulthood suggesting interplay between maternal nutrition and regional fetal fat tissue development. To elucidate the underlying mechanisms, we compared the genomic expression profiles (Illumina® microarrays) of preadipocytes from omental (OM) and subcutaneous (Sc) abdominal and femoral (ScA, ScF) fat depots between baboon fetuses from mothers fed control and 30% nutrient-restricted diets (CTR vs. MNR; n=4/group). We found 118 genes differentially expressed among depots and/or between maternal diets and 62 of them were measured by RT-PCR. Levels of five gene transcripts were significantly higher (1.3-3.7 fold) in MNR than in CTR fetuses in all depots—ETV4 (upstream inducer of PPARγ); SFRP1 (inhibitor of adipogenesis-inhibitory Wnt-β catenin signaling); TP53BP2 (activator of adipocyte-protective and lipogenic p53 signaling); α8 subunit of integrin receptor (ligands fibronectin and vitronectin regulate preadipocyte proliferation, migration, and adipocyte cluster formation); and gap junction protein GJC2 (unknown expression in fat)—suggesting precocious preadipocyte differentiation . Several genes were differentially expressed in MNR vs. CTR fetuses in a depot-specific way—lower levels of LPS1 (facilitates PKCβI-dependent activation of ERK cascade, a stimulator of preadipocytes proliferation) in Sc depots and CD248 (found in vasculogenesis-promoting perivascular

cells) in ScF depot, and higher levels of CSMD2 (a tumor suppressor gene) in ScA preadipocytes—suggesting superimposed impaired preadipocyte proliferation and vasculogenesis in Sc, primarily in ScF, depots. These changes may hamper attainment of adequate progenitor pool and contributes to postnatal limited adipogenic potential and predisposition to central adiposity.

052

PRENATAL AD.VEGF GENE THERAPY INCREASES FETAL GROWTH VELOCITY IN THE ABSENCE OF A MEASURABLE EFFECT ON UTERINE BLOOD FLOW IN AN OVINE PARADIGM OF FETAL GROWTH RESTRICTION

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Introduction: Adenovirus (Ad) mediated over-expression of vascular endothelial growth factor (VEGF) in the uterine arteries (UA) increases uterine blood flow (UBF) in normal sheep pregnancy1. Ad.VEGF therapy increases fetal growth velocity and term birthweight in growth-restricted fetuses of overnourished adolescent dams2. Herein the effects of Ad.VEGF on UBF, growth velocity and fetal body/organ weights at 0.9 gestation were investigated.

Methods: Singleton pregnancies were established in 57 adolescent ewe-lambs using embryo transfer. Dams were overnourished to generate placental and fetal growth restriction or fed a control diet. At 89±1.5d gestation, overnourished ewes were randomised to receive 1x1012 particles Ad.VEGF (n=18), Ad.LacZ (n=14) or saline (n=13) injected into each UA at laparotomy. Controls received saline only (n=12). Transonic® flow probes were fitted around the UA supplying the gravid horn. Fetal biometry and wellbeing were assessed blind by weekly ultrasound (single operator). UBF was monitored on alternate days until necropsy at 131±1.6d.

Results: Relative to controls, UBF was reduced in Ad.LacZ/saline but not Ad.VEGF groups between 92±1.5d and 96±0.4d (p=0.004–0.047). Thereafter no significant differences were demonstrated. Fetal abdominal circumference (AC) was greater in Ad.VEGF versus Ad.LacZ/saline groups at 112±0.1d gestation, 22±0.2d post-injection (p=0.047/0.031) and 119±0.2d gestation, 29±0.2d post-injection (p=0.016/0.032). Biparietal diameter (BPD):AC ratios were lower in Ad.VEGF versus Ad.LacZ/saline groups from 112±0.1d onwards (p=<0.001–0.002). Necropsy weight was not significantly different between Ad.VEGF/Ad.LacZ/saline groups (4395±188g/4153±202g/4008±234g) but was reduced in all grorups relative to control-intake fetuses (5084±124g, p=0.001–0.016). Fewer fetuses weighed two standard deviations or more below the control mean in Ad.VEGF versus Ad.LacZ+saline groups (5/18 versus 18/28, p=0.038). Relative brain weight and brain:liver weight ratios were lower in Ad.VEGF versus Ad.LacZ+saline groups (p=0.009/0.046, respectively).

Conclusion: Ad.VEGF significantly increased fetal growth velocity at 0.77–0.82 gestation with evidence of an attenuated "brain sparing" effect, but had no measurable effect on UBF.

(1) David AL, Torondel B, Zachary I et al. Local delivery of VEGF adenovirus to the uterine artery increases vasorelaxation and uterine blood flow in the pregnant sheep. Gene Ther 2008; 15: 1344-50
(2) Carr DJ, Aitken RP, Milne JS, Peebles DM, Martin J, Zachary I, Wallace JM, David AL. Maternal Ad.VEGF gene therapy increases fetal growth in growth-restricted sheep fetuses. BJOG 2011 (in press)

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TOMATO JUICE PROTECTS THE LUNGS OF THE OFFSPRING OF FEMALE RATS EXPOSED TO NICOTINE DURING GESTATION AND LACTATION.

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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 study the long term effect of maternal nicotine exposure on the structural integrity of the lungs of the offspring, and secondly to determine whether supplementing the mother's diet with tomato juice, as a rich source of antioxidants such as lycopene, will prevent the effects of nicotine on the lungs of the offspring. Wistar rats were used in the study. After mating the rats were randomly divided into 4 groups. One group received nicotine (1 mg/kg body weight/day); a second group received tomato juice while a third group received nicotine and tomato juice. The controls receive saline. Morphological and morphometric techniques were used to evaluate changes in the lung structure of the offspring at postnatal days 21, 42, 63 and 84. Neither nicotine, nor tomato juice had any effect on the growth of the offspring. Although maternal nicotine exposure during gestation and lactation had no effect on the lung parenchyma of the offspring up to weaning, deterioration and other structural changes started to appear around postnatal day 42, that is, 3 weeks after weaning and thus the onset of nicotine withdrawal. Microscopic emphysema was apparent at postnatal day 42, the increase in male and female lung volume from postnatal day 63 and thickening of the alveolar walls at postnatal day 84. All these nicotine-induced structural changes were prevented by supplementing the mother's diet with tomato juice.

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THE EFFECTS OF MATERNAL HIGH FAT FEEDING ON BODY FAT MASS AND SUSCEPTIBILITY TO DIET INDUCED OBESITY CAN BE REVERSED BY INTERVENTIONS DURING THE NEONATAL PERIOD

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Background: There is growing evidence that perinatal exposure to excessive nutrient supply contributes to an increased risk of obesity in the offspring. However, it is unclear whether and to what extent the effects of prenatal exposure to an excess nutrient supply can be reversed.

Objective: To determine if the effect of maternal high-fat feeding on fat mass and susceptibility to diet induced obesity in the offspring could be prevented by cross-fostering the pups onto a Control dam at birth.

Methods: Albino Wistar dams were fed either standard rat chow (Control, n=11) or high-fat cafeteria diet (HF, n=10) for 4 weeks before pregnancy and during pregnancy and lactation. All offspring were cross-fostered onto a dam from either from the same or different treatment group and were fed standard rat chow after weaning (3 weeks of age). At 3 months of age, all offspring were provided with free access to a high-fat cafeteria diet. Body fat mass was determined at 3 week, 6 weeks and 4 months of age.

Results: HF pups suckled by another HF mother (HF-HF) had a higher fat mass at weaning (HF-HF, 0.52 ± 0.007%; C-C, 0.35 ± 0.003%, $P<0.0001$), and this effect was reversed by cross-fostering the HF offspring onto a Control dam (HF-C, 0.25 ± 0.004% vs 0.35 ± 0.003%, P=ns). Pups exposed to the HF diet during the suckling period, but not HF offspring cross-fostered onto a Control dam at birth, exhibited an increased preference for fat and sugar intake at 3 months of age ($P<0.001$), and an increased percentage body fat after 4 weeks on a HF diet ($P<0.001$).

Conclusions: Our findings demonstrate that the effects of exposure to a HF diet during fetal life can be reversed by removing the HF stimulus during the suckling period. These data highlight the potential for reversing the the effects of prenatal exposure to HF diets by interventions applied after birth.

POSTER

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ACTIVIN: TOWARDS A NEW MOUSE MODEL OF PREECLAMPSIA

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Background: Circulating levels of activin are increased 10-fold in women with preeclampsia compared to women with a healthy pregnancy, with increased levels apparent months before the clinical onset of the disease. Activin can also induce endothelial dysfunction in vitro and follistatin – the activin binding protein – can block the actions of preeclamptic serum on endothelial cells in vitro. These data suggest that activin may cause preeclampsia. *Aim*: To develop an in vivo model of preeclampsia by administering activin to pregnant mice. *Methods*: Pre-mating, 6-8 week old C57Bl6 female mice underwent surgery for implantation of a miniaturized telemetric blood pressure probe (Datasciences International PA-C10) into the left carotid artery. On day 10 gestation, pregnant mice underwent further surgery to implant an osmotic minipump to deliver either saline (controls) or activin A (total 5ug to deliver 3.6ug/kg/day). BP was measured on day 8, 12, 14, 16. Urine, for proteinuria, was collected on day 16. All animals were culled on dy18, allowing collection of maternal blood by cardiac puncture for activin, 8-isoprostane, urate, creatinine, AST, ALT, LDH, platelets. Organs and blood vessels were collected for histopathology, mRNA, wire myography, and assessment of oxidative stress and pro-oxidant systems. *Results*: Activin administration increased circulating activin 3-4 fold (mean±SEM 2095±169 vs 735±204pg/mL, $P<0.001$). Activin treatment increased endothelial oxidative stress (detected by dihyroethidium staining), increased systolic BP ($P=0.01$), increased urinary albumin:creatinine ratio ($P<0.01$), increased LDH and AST ($P<0.001$), and decreased average pup birthweight ($P<0.0001$). *Conclusion*: The administration of activin to pregnant C57Bl6 mice recapitulates many of the features of human preeclampsia. This model offers opportunities to explore causative pathways and to develop novel therapies targeting those pathways.

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DOES DAILY ETHANOL EXPOSURE DURING LATE OVINE GESTATION ALTER FETAL LIVER DEVELOPMENT?

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Background: Chronic, high-dose maternal alcohol (ethanol) consumption during pregnancy can affect liver development and cause liver injury, including cirrhosis, in the offspring. Previous studies have reported increased susceptibility to liver infection after high-dose ethanol exposure in adult rodents. The effects of daily ethanol exposure on the fetal liver have not been characterised. Aim: To determine the effects of daily ethanol exposure in late gestation on the morphology, inflammatory status, and mRNA expression of the iron regulating hormone hepcidin, of the ovine fetal liver. Methods: Pregnant ewes were chronically catheterised at 90 days of gestation (DG; term ~145DG) for the daily i.v. infusion of ethanol (0.75g/kg, n=9) or saline (n=8) over 1h from 95-133DG. At necropsy (134DG) fetal livers were collected for histological and molecular analyses. Tissue sections were stained with hematoxylin and eosin, and Oil Red O to assess general liver morphology and lipid deposition, respectively. Cell proliferation (anti-Ki67), ferric iron deposition (Perls' Prussian blue) and perivascular collagen (Picrosirius Red) were quantified. Interleukin (IL)-1β, IL-6, IL-8, tumour necrosis factor (TNF)-α and hepcidin mRNA levels were determined using quantitative real-time PCR. Results: Body and liver weights and general liver morphology were not different between ethanol-exposed and control fetuses. There was no lipid deposition evident in any fetal livers. There were no differences in cell proliferation, perivascular collagen deposition, and IL-1β, IL-6 or IL-8 mRNA levels between groups. However, there were significant decreases in liver iron deposition and hepcidin and TNF-α mRNA levels following ethanol exposure compared with controls (all $p<0.05$). Conclusions: Daily ethanol exposure during the third-trimester-equivalent in sheep does not alter fetal liver morphology; however, decreased fetal liver ferric iron content and hepcidin gene expression indicate that iron homeostasis is altered. Reduced pro-inflammatory cytokine expression has also been observed in other tissues, which suggests that innate immunity may be altered.

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SEX-SPECIFIC STRUCTURAL MATURATION OF THE MYOCARDIUM IN PRETERM PIGLETS

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Introduction: Following preterm birth up to 30% of neonates fail to maintain sufficient systemic blood flow resulting in increased morbidity and mortality. This may result from an immature myocardium failing to adapt to increased vascular resistance. Poor adaptation is more common in male preterm infants than in females.

Methods: Preterm piglets were used to investigate sex-specific aspects of th e maturation of cardiac myocytes in terms of volume and nucleation, apoptosis and proliferation, and the histology of the myocardium. P iglets were delivered by caesarean section at 92/115 days or at term. An additional preterm group was exposed to maternal glucocorticoid treatment.

Results: In p reterm piglets, body and heart weight were approximately half that of term piglets. In male preterm piglets, heart weight as percentage of body weight was lower than at term. The proportion of cardiac myocytes that were binucleated was lower in preterm compared to term piglets, and m yocytes were smaller than at term. There were no sex differences in the ratio of uni:bi-nucleated myocytes but right ventricular myocytes were larger in male preterm piglets.

Glucocorticoid exposure was associated with an overall increase in body and heart mass exclusively in female piglets, and a reduced relative right ventricular mass in both male and female preterm piglets. In female piglets only, glucocorticoid exposure was associated with an increased proportion of binucleated myocytes in the right ventricle. Left ventricular myocyte volumes were greater in glucocorticoid exposed piglets.

Conclusions: The smaller size of the heart relative to the body in male preterm piglets may contribute to reduced ability of the preterm heart to adapt to the post natal circulation. Maternal glucocorticoid treatment promotes growth and structural maturity of the myocardium to a greater degree in females than in males. Differences in structural maturity may contribute to the male disadvantage seen in preterm infants.

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LONG TERM BEHAVIOURAL OUTCOMES OF CHRONIC LOW DOSE PRENATAL ETHANOL EXPOSURE

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Introduction: Excessive alcohol consumption during pregnancy can lead to a wide spectrum of disorders and defects in offspring, which are collectively referred to as Foetal Alcohol Spectrum Disorders. However, recent evidence suggests that mild alcohol consumption during pregnancy may not have detrimental effects on cognition and behaviour of the offspring. The aim of this study was to examine the effect of exposure to low dose ethanol during gestation on behavioural changes in adult and aged offspring.

Methods: Female Sprague Dawley rats were fed a liquid diet containing a low dose of ethanol (6% v/v, Ethanol) or a calorie matched control diet for the duration of pregnancy (Control). Male (Adult n=15, Aged n=17, Control; Adult n=13, Aged n=12, Ethanol) and female (Adult n=12, Aged n=14, Control; Adult n=10, Aged n=13, Ethanol) offspring were tested at 6-9 months (Adult) and 15-18 months (Aged) of age to assess a number of behavioural domains including anxiety, exploration, sensorimotor gating and spatial memory, as well as ethanol preference.

Results: Prenatal exposure to a low dose ethanol diet resulted in a subtle, behavioural phenotype affecting aspects of anxiety at 6-9 months of age ($P=0.06$) but not at 15-18 months. There was no effect of prenatal treatment on locomotion, sensorimotor gating, spatial memory or ethanol preference at either age ($p>0.05$).

Conclusions: This data suggests that exposure to low dose ethanol during early neural development may not lead to long lasting behavioural changes in adult life.

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DOES ENDOTOXIN EXPOSURE DURING LATE GESTATION CAUSE BRAIN INJURY?

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Background: Intrauterine inflammation is an independent risk factor for Cerebral Palsy (CP) in near-term infants[1]. Currently there is no effective therapy to treat brain injury in infants with CP with the exception of hypothermia in term babies[2]. The aim of this study is to establish a model of brain injury induced via intrauterine inflammation during late gestation in the preterm sheep in order to then test therapeutic strategies.

Method s : Pregnant ewes underwent surgery at 123 days of gestation (DG, term ~147 DG) to implant vascular and amniotic catheters. High (40 m g/day; n=2) or low dose (5 m g/day; n=2) lipopolysaccharide (LPS), or saline (n=1) was infused continuously from 127 to 133 DG via the fetal femoral vein. Fetal blood pressure (BP) and heart rate (HR) were recorded and blood gases were measured. The lambs were delivered at 134 DG and necropsy was performed at 140 DG. Brains were perfusion fixed and stained with H&E and thionin for gross structural assessment. Immunohistochemistry was performed using antibodies directed against ionized calcium binding adapter molecule 1 (Iba-1), glial fibrillary acidic protein (GFAP), myelin basic protein (MBP) and neurofilament 200 (NF200) for the quantitative assessment of microglia, reactive astrocytes, mature myelin, and axons respectively

Results : In the high dose group, there appeared to be an increase in HR, $PaCO_2$ and lactate; there was no apparent difference in BP in any group. There was no evidence of overt injury, and no apparent difference in the cellular density of microglia or astrocytes, or in the optical density of MBP or NF200 between groups.

Conclusions : These preliminary studies show that continuous infusion of LPS in late gestation may cause some physiological changes in the fetus but no structural alterations in the brain after preterm birth. Thus the late gestation ovine brain appears to be resistant to effects of intrauterine inflammation.

(1) Wu YW et al. Chorioamnionitis and cerebral palsy in term and near-term infants. Jama. 2003;290: 2677-2684
(2) Gluckman PD et al. Selective head cooling with mild systemic hypothermia after neonatal encephalopathy: multicentre randomised trial. Lancet. 2005;365: 663-670

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HISTOLOGICAL, PHENOTYPIC AND PROLIFERATIVE POTENTIAL OF HEMATOPOIETIC CELL POPULATIONS IN THE FETAL, NEONATAL AND ADULT SPINY MOUSE

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Background: Immunological competence at the time of birth is highly variable amongst mammalian species and the ability to mount an effective immune response at birth is limited to species where the immune organs are sufficiently mature, structurally and functionally. We hypothesised that, as for the other major organs, the immune system of the spiny mouse would be relatively mature at birth, consistent with its precocial mode of development, compared to that of the mouse, a closely related species that follows an altricial mode of development. Methods: The structure of the spiny mouse immune system was examined histologically throughout gestation, at the time of birth, and at sexual maturity and compared to that of C57BL/6 mice. Splenocytes were isolated and their proliferative potential, by stimulation with PMA + ionomycin and LPS, was determined for both species at birth and at sexual maturity. CD45R positive leukocyte populations were isolated and characterised in the fetal, newborn and adult spiny mouse liver, lymph, bone marrow, spleen and thymus. Results: The thymus and spleen showed structural maturity at ~0.7 gestation in the spiny mouse, compared to after birth in the mouse. Splenocytes from term fetal and day of birth spiny mice showed a greater in vitro proliferative response compared to that in aged matched mice. The number and fluorescence intensity of CD45R positive cells increased with age in all spiny mouse tissues examined, except the liver, where only a small population was identified and where cell number did not change with age. Conclusion: The spiny mouse immune system is structurally and functionally more mature at birth compared to that of the mouse. The observed presence of CD45R leukocytes in many organs is similar to that of other mammalian species. Ongoing work is characterising other hematopoietic cell populations in this species.

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DOES HIGH DOSE CHRONIC CAFFEINE TREATMENT AFFECT PHYSIOLOGICAL STATUS AND BRAIN DEVELOPMENT OF THE IMMATURE FETUS?

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Background: The current treatment for apnoea of prematurity (AOP), a common condition in very preterm infants, involves a loading dose of 20mg/kg caffeine (citrate) followed by a daily maintenance dose of 5-10mg/kg. As this regimen is not always sufficient to abolish apnoea [1] there is mounting pressure on clinicians to use higher doses. However, the effects of higher doses of caffeine on the developing brain have not been subject to rigorous neuropathological evaluation in a suitable animal model. Our aim is to determine the effects of high dose caffeine exposure on the immature brain and blood chemistry. Methods: A high dose caffeine regimen (50mg/kg loading; 40mg/kg daily maintenance dose; citrate equivalent, n=6) or an equivalent volume of saline (n=6) was administered to the fetus via the maternal circulation daily from 104 to 118 days of gestational age (DGA). Fetal and maternal blood was sampled for the assessment of blood chemistry and plasma caffeine concentrations, from 104-118DGA. At necropsy (119DGA) fetuses were weighed and body dimensions measured. Fetal brains were perfusion-fixed and processed for structural analysis. Results: There were no significant differences between groups in fetal arterial blood chemistry (pH, PCO2, PO2, SaO2, tHb, glucose or lactate) throughout the treatment period. There was no significant difference in body weight or body dimensions between the two groups. Fetuses exposed to high dose caffeine tended to have heavier brains when brain to body weight ratio was assessed ($p=0.06$). There was no significant difference in the percentage of white matter (WM) occupied by microgliosis in caffeine compared to control brains. Conclusions: High-dose caffeine exposure does not affect fetal blood chemistry and does not result in microgliosis in the developing WM. Further neurostructural analysis is necessary to explore and determine potential effects of high dose caffeine on the immature brain.

(1) Schmidt, B., et al., Caffeine therapy for apnea of prematurity. N Engl J Med, 2006. 354(20): p. 2112-21.

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CHRONIC EXPOSURE TO TNF-A IN AN *IN VITRO* OVINE MODEL OF PRETERM BRAIN INJURY: EFFECTS ON GLUTAMATE RECEPTORS

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Intrauterine infection and inflammation are highly associated with white matter injury (WMI) and neurological impairment in preterm infants. Inflammatory mediators produced in response to infection, in particular tumour necrosis factor alpha (TNF-α), are strongly associated with the pathogenesis of oligodendrocyte (OL) death; however, the precise pathways involved are poorly understood. Excitotoxicity is caused by over-activation of glutamate receptors (GluRs) leading to cell death. We hypothesise that a link between inflammatory signalling and release of glutamate from astrocytes and microglia, potentially exacerbated by a phenotypic change in OL-localised GluR subunits, may lead to a cell more susceptible to injury.

We investigated AMPA (GluR1-4) and NMDA (NR1) receptor subunit expression at the mRNA and protein levels in response to chronic TNF-α exposure in a primary mixed glial cell culture over 5 days.

Preliminary results demonstrate that across the time course studied, mRNA expression for the GluR2 subunit remained largely unchanged, whereas GluR1, 3 and 4 were significantly altered at varying time-points. Also, NR1 mRNA expression progressively increased and remained elevated at later time-points while apoptotic OLs were observed from 24 hours following onset of TNF-α exposure.

Our data of TNF-α induced changes in expression of GluRs *in vitro* suggest that glutamate may contribute to WMI in response to infection/inflammation. Further incorporation of GluRs antagonists may lead to a better understanding of the glutamate and GluR subunit response to infection/inflammation and their role in the genesis and development of preterm WMI.

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INCREASED CELL PROLIFERATION AND ANGIOGENESIS IN THE FETAL SUBVENTRICULAR ZONE IS CORRELATED WITH THE SEVERITY OF GROWTH RESTRICTION.

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Background: Adverse prenatal factors can result in abnormal brain development, contributing to the aetiology of several neurological disorders. Intrauterine insults could occur during neurogenesis and gliogenesis, disrupting these events. Here we investigate the effects of chronic placental insufficiency (CPI) on cell proliferation and the microenvironment in the subventricular zone (SVZ) of the brain.

Methods: At 30 days of gestation (dg; term~67dg), CPI was induced in pregnant guinea pigs via unilateral uterine artery ligation to produce growth-restricted (GR) fetuses (n=7); controls (n=6) were from the unoperated horn. At 60dg, fetal brains were stained immunohistochemically to identify proliferating cells (Ki67), immature neurons (PSA-NCAM), astrocytes (glial fibrillary acidic protein, GFAP), microglia (Iba-1) and the microvasculature (von Willebrand Factor) in the SVZ.

Results: There was no difference ($p>0.05$) in the a) number of Ki-67- immunoreactive (IR) cells, b) density of Iba-1-IR microglia or c) percentage of SVZ occupied by blood vessels in control versus GR fetuses. Regression analysis revealed that the a) number of Ki67-IR cells and b) percentage of SVZ occupied by blood vessels, increased ($p<0.05$) with the severity of growth restriction. The percentage of SVZ occupied by blood vessels was also correlated ($p<0.05$, $r^2=0.61$) with the number of Ki67-IR cells in the SVZ in GR fetuses. PSA-NCAM-IR was present in the SVZ in control and GR brains, whereas GFAP-IR was negligible.

Conclusion: CPI increases cell proliferation and promotes angiogenesis in the fetal SVZ when growth restriction is severe. These proliferating cells are likely to be neurons; their long-term survival is being assessed. Furthermore, the microvasculature within the SVZ influences cell proliferation.

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EFFECT OF CHRONIC LOW DOSE ALCOHOL EXPOSURE *IN UTERO* IN THE HEARTS OF RAT OFFSPRING

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Introduction: Epidemiological studies demonstrate that individuals may be 'programmed' to develop cardiovascular disease as a result of insults that occur to the fetus in utero. Specifically, high alcohol consumption throughout pregnancy is known to affect cardiomyocyte growth and maturation leading to permanent adverse structural changes in the heart with long-term deleterious effects in cardiac function. We aimed to determine whether low levels of maternal alcohol consumption is also detrimental to cardiomyocyte development and number in hearts of rat offspring. Methods: Pregnant Sprague-Dawley rat dams were fed a control diet of 6% (volume/volume) liquid-based ethanol supplemented (isocaloric) diet throughout gestation. At embryonic day 20, expression of genes involved in cardiac development was analysed. Whilst at postnatal day 30, cardiomyocyte number in the left ventricle and adjoining septum was determined stereologically. Results: There was a significant ($p=0.012$) down-regulation of insulin-like growth factor 1 (IGF1) mRNA expression, but no differences were observed in IGF2, IGF1R or AT1bR levels which are also associated with cardiac growth. Similarly, the expression of apoptotic genes, such as Bax, Bcl-2 and p53 remained unaltered. However, the decrease in IGF1 gene expression levels did not appear to adversely affect heart growth, with no significant differences seen in heart weights or heart wall volume, or cardiomyocyte number in male or female offspring. Conclusions: Overall, our results indicate that maternal consumption of alcohol at low levels during pregnancy is not detrimental to growth of the fetal heart. This suggests that effects of prenatal alcohol exposure on heart growth are dose dependent. This preliminary data is encouraging given the high proportion of pregnant women who drink chronically low levels of alcohol throughout gestation.

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DIFFERENTIAL EFFECTS OF EPIDERMAL GROWTH FACTOR ON THE INFLAMMATORY RESPONSE ALONG THE DEVELOPING HUMAN INTESTINE.

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Background: The occurence of many neonatal inflammatory intestinal diseases in preterm infants highlights the susceptibility of the immature intestine to respond inadequately to nutrients and microbes. Several lines of evidence suggest an important interrelationship between Epidermal Growth Factor (EGF) and intestinal inflammation. However the molecular mechanisms underlying the beneficial effects of EGF on the pathogenesis on inflammation remain to be clarified.

Objectives: The purpose of this study was to evaluate the role of EGF on the gene expression profiles of human developing ileum and colon tissues at mid-gestation in serum-free organ culture using cDNA microarrays. Methods: We compared the gene expression profiles of cultured human fetal small and large intestinal explants in the absence or in the presence of 50 ng/mL EGF using a cDNA microarray and analyzed the data with the Ingenuity Pathway Analysis software (IPA).

Results: We found that a significant proportion of genes were differentially expressed in the two segments. IPA functional analyses revealed that EGF, in addition to modulating different cellular, molecular and physiological functions in each segment, modulated the inflammatory response in both intestinal segments in a distinct manner. Several intestinal-derived chemokines such CXCL10, CCL14 and CXCL5 were differentially regulated by EGF along the developing human intestine, while CCL25 and TFF1 were similarly regulated by EGF in both intestinal segments.

Conclusion: Although the findings presented here suggest a mechanistic basis for the beneficial effects of EGF in intestinal inflammation, they also establish that the location of the intestinal injured area should be considered when attempting to define the tissue-specific effects of EGF in the inflammatory response.

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URINARY CONCENTRATING DEFECT IN RESPONSE TO EXOGENOUS ARGININE VASOPRESSIN INFUSION AND WATER DEPRIVATION IN MALE SHEEP FOLLOWING FETAL UNINEPHRECTOMY

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Fetal uninephrectomy (uni-x) in male sheep at 100 days of gestation (term=150days) results in reduced glomerular filtration rate (GFR) and elevated mean arterial pressure (MAP) at 6 months of age. This study investigated whether this reduction in renal function was associated with impaired urine concentrating ability of the remnant kidney by examining responses to 1) non-pressor dose of exogenous arginine vasopressin (AVP (0.2 µg/kg/h i.v.) and 2) 30 hours of water deprivation and whether responses worsened with age in conscious animals. Basal MAP was higher in uni-x animals at both ages compared to sham group and increased more with ageing in uni-x group (6mth: 8 ± 3 mmHg, 4 y: 12 ± 2 mmHg, $P_{group}<0.001$, $P_{groupxage}<0.01$). Basal GFR was lower in uni-x compared to sham group at both ages (6 mth: 26%; 4 y: 33%, $P_{group}<0.001$). GFR declined with age in both groups but the decrease was greater in uni-x animals (sham: 16%, uni-x: 26%; $P_{age}<0.001$, $P_{groupxage}<0.001$). Basal gene expression of vasopressin-2 receptor (V2R) and aquaporin-2 (AQP2) water channel was lower in uni-x animals at both ages ($P<0.01$). In response to AVP infusion, urine osmolality increased in both treatment groups, but the response was significantly lower in uni-x animals and became further reduced with ageing ($P_{age}<0.001$). Similarly in response to water deprivation, the decrease in UFR and the increase in urine osmolality was lower in uni-x animals at both ages compared to sham group ($P_{group}<0.001$). Plasma AVP levels increased similarly in both treatment groups in response to water deprivation. Since plasma AVP levels increased similarly in both treatment groups in response to dehydration, the impaired urine concentrating ability of uni-x animals is likely associated with the reduced renal gene expression of AQP2 and V2R. Thus a low nephron endowment increases the risk of hypertension and chronic renal disease which becomes exacerbated with ageing and may incur greater vulnerability to physiological challenges such as water deprivation as observed in uni-x animals.

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CHANGES IN FETAL SKIN MORPHOLOGY, PERMEABILITY AND *TGM1* GENE EXPRESSION FOLLOWING MATERNAL GLUCOCORTICOID EXPOSURE IN THE MOUSE

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Preterm infants possess an immature epidermal barrier that predisposes to dehydration and evaporative heat loss[1]. There is evidence to suggest that glucocorticoids (GC) may accelerate epidermal barrier maturation[2-3] however relatively little is known regarding any differential effects of natural and synthetic GC. This study describes the effects of maternal GC exposure on murine skin development and compares the effects of natural (corticosterone, CORT) and synthetic GC (dexamethasone, DEX).

Pregnant C57BL/6 mice were administered DEX (1µg/kg/h), CORT (33µg/kg/h) or 0.9% saline (SAL) for 60 hours via osmotic mini-pump beginning at embryonic day (E) 12.5. At E17.5 animals were euthanased, fetuses weighed, and samples of ventral skin collected for analysis. Skin morphology and epidermal thickness were evaluated using sections stained with haematoxylin and eosin. Expression of *Nr3c1, Tgm1, Flg, Lor* and *Ivl* was examined by qRT-PCR. A toluidine blue assay was used to assess epidermal permeability. The extent of staining was scored on a scale of 0 (no penetration) to 5 (penetration across all body regions).

The ventral epidermis of mice exposed to CORT was thinner (37.05 ± 7.51µm; mean ± SD) than the SAL-treated group (45.60 ± 2.61µm; $P<0.05$) however CORT-treated mice were significantly smaller than those in the other groups ($P<0.05$). CORT-treated mice also demonstrated a relatively poor barrier to dye penetration compared to the SAL group ($P<0.05$). Skin from CORT-treated animals showed higher *Tgm1* ($P<0.05$) expression compared to the SAL group. While trends were observed in the DEX group no statistically significant differences were found.

This study has demonstrated changes in epidermal thickness, permeability and skin *Tgm1* expression following excess maternal GC exposure. Morphological changes observed in CORT-treated fetuses may in part be due to their smaller body size. Analysis of sex specific treatment interactions may further elucidate the effect of maternal GC exposure on the developing skin.

(1) Rutter N & Hull D (1979) Arch. Dis. Child. 54:858-868.
(2) Aszterbaum et al. (1993) J. Clin. Invest. 91:2703-2708.
(3) Patel et al. (2006) Proc. Natl. Acad. Sci. USA 103:18668-18673.

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DO CONVALESCENT PRETERM INFANTS EXHIBIT HEART RATE VARIABILITY?

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Background: Beat-to-beat heart rate fluctuations are associated with normal physiological regulatory processes, and decreased heart rate variability (HRV) has been associated with adverse outcomes in both infants and adults. The literature on HRV in preterm infants, and how it develops with maturation is limited. We therefore examined this in six convalescent preterm infants (three male) born < 32 weeks gestational age (GA) at 32-34 weeks postmenstrual age (PMA) and again two weeks later. Methods: ECG and nasal pressure recordings were made during sleep after a feed. We extracted heart rate and respiratory time series from active sleep (AS) and quiet sleep (QS) recorded with the infant in the supine position at the two time periods. Results: Median GA at birth was 29.5 weeks (range 24-31 weeks) and median birthweight 1144 g (range 778-1630g). Initial recordings were at 32 weeks PMA for two infants, 33 weeks PMA for three infants and 34 weeks PMA for one infant. Heart rate did not change significantly with sleep state, or with time, but the coefficient of variation of heart rate (a measure of HRV) was greater in AS than in QS at both ages. The most common characteristic of HRV was 10-15sec periodic fluctuations with an amplitude of 200ms, observed in 16 of 24 recordings. In the remaining 8 cases we observed episodic cardiac decelerations, in most cases associated with preceding respiratory pauses. Very small amplitude fluctuations in heart rate at the respiratory frequency were detected in 3 of 24 recordings. Conclusions: The most common pattern of HRV in premature infants was a 10-15 second periodicity, and this did not change with sleep state or with 2 weeks of maturation. Respiratory mediated fluctuation in heart rate, reported by other authors as a significant feature of infant HRV, was rarely observed.

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IS THE MALE DISADVANTAGE IN RESPIRATORY OUTCOME FOLLOWING PRETERM BIRTH A RESULT OF ALTERED SURFACTANT COMPOSITION?

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Background: Male preterm infants are at a greater risk of respiratory morbidity and mortality than females. The mechanisms responsible for this "male disadvantage" in respiratory function are unknown. Previous studies in our lab have shown no differences in lung structure between male and female preterm lambs. It has been suggested that pulmonary surfactant composition in male preterm infants differs from that in females, contributing to poorer respiratory function.

Aims: To determine whether there are differences between male and female preterm lambs in (a) surfactant protein (SP) mRNA levels in lung tissue and (b) phospholipid (PL) composition in amniotic fluid and bronchoalveolar lavage fluid (BALF).

Methods: At ~125 days of gestation (DG; term ~147DG), 9 female and 10 male fetal sheep underwent surgery for catheter implantation. Ewes received betamethasone (5.7mg, im) at 131DG. Amniotic fluid was sampled at 131 and 133DG. At 133DG, lambs were delivered via caesarean section, monitored under conditions of spontaneous breathing for 4h and euthanized. Lung tissue and BALF were collected for analysis. Liquid chromatography mass spectrometry and qPCR were used to determine PL composition in amniotic fluid and BALF, and SP mRNA levels in lung tissue, respectively.

Results: No significant differences were observed between males and females in PL composition in amniotic fluid, and in lung SP mRNA levels. In BALF, males had a significantly lower percentage of total phosphatidylcholine (PC, the major PL in surfactant; 68.0±1.5% vs 72.6±1.1%, $p<0.05$) and PC32:0 (DPPC, the major PC species; by ~10%, $p<0.05$) compared to females. The percentages of PC34:2 and PC36:2 were greater in males by ~20% and ~35% respectively ($p<0.05$).

Conclusions: The lower proportion of total PC and DPPC in preterm males could contribute to their poorer respiratory function. Higher level of PC34:2 suggests increased vascular permeability in male lungs.

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OSTEOCHONDRITIS DISSECANS (OCD) IN AUSTRALIAN THOROUGHBRED HORSES IS ASSOCIATED WITH CHRONIC HYPOINSULAEMIA IN EARLY POST-NATAL LIFE.

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Background: OCD is a common developmental orthopedic disease in young horses. Previous work has examined post-natal nutrition as a causal factor in equine OCD, and OCD has indeed been associated with insulin resistance in yearling horses (Ralston, 1996). However we postulate that fetal programming and maternal diet may be important in the pathogenesis of OCD. The aim of this study was to examine the relationship between glucose homeostasis and the occurrence of OCD in Australian Thoroughbred horses.

Methods: Jugular blood samples, following an overnight fast in stables, were obtained from yearlings at Thoroughbred stud farms in the Hunter Valley region (NSW) across two breeding seasons. OCD and other skeletal abnormalities were determined via radiographic analysis by experienced veterinary clinicians, and categorized as high-risk abnormalities (OCD and related subchondral bone cysts, fractures or lesions) versus no significant abnormalities (NSA). Plasma insulin and glucose concentrations were determined by RIA and Hexokinase method respectively.

Results: Overall the incidence of high-risk bone abnormalities across sampled studs was 23.2%. Fasting plasma insulin concentrations were significantly ($P<0.01$) lower in yearlings within the high-risk group (2.3±0.2 mIU/L; n=42) compared to NSA group (3.4±0.2 mIU/L; n= 91). However fasting glucose concentrations were not significantly different between groups. There was no significant effect of stud farm or breeding year on overall insulin or glucose results. Furthermore, resting insulin concentrations from samples obtained earlier in post-natal life, at foal and weanling ages (3 and 6 months), revealed significant hypoinsulinaemia in horses that subsequently developed OCD and related bone abnormalities by yearling age.

Conclusions: The results from Australian Thoroughbred horses suggest an association between OCD and chronic hypoinsulinaemia in early post-natal life. This would support the notion that the pathogenesis of equine OCD is related to development in utero.

(1) Ralston, S.L. (1996) Hyperglycemia/hyperinsulinemia after feeding a meal of grain to young horses with osteochondritis dissecans (OCD) lesions. Pferdeheulkunde 12: 320-322.

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ALTERED CARDIOVASCULAR AND NEURAL RESPONSES OF THE PRETERM FETUS TO ASPHYXIA AFTER CHRONIC EXPOSURE TO LPS

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Exposure to infection can increase asphyxial injury. This study assessed whether LPS-sensitisation is due to compromise of fetal cardiovascular adaptations to asphyxia.

Chronically-instrumented preterm fetal sheep received either LPS as a continuous low dose infusion *plus* 3 1µg boluses of LPS or saline. Asphyxia was induced for 15min by umbilical cord occlusion (UCO) on day 5. Fetuses were euthanised on day 10 and brains taken for histology. Fetal heart rate (HR), blood pressure (BP), femoral blood flow (FBF) and conductance (FVC) and carotid blood flow (CaBF) and conductance are presented as mean ± SE.

In LPS fetuses vs. controls, HR was higher in the baseline ($p<0.05$), fell faster at the onset of UCO ($p<0.05$), and was lower during UCO (min 3-10, $p<0.05$). BP was lower in the baseline ($p<0.05$), and higher during UCO (min 3-10, $p<0.05$). FBF and FVC were not different in the baseline, but higher from 10-15min of UCO ($p<0.05$). CaBF and CaVC were higher in the baseline ($p<0.001$). During occlusion CaVC fell rapidly and remained significantly lower than controls ($p<0.05$) whereas CaBF was not different from controls. During UCO, lactate levels were highly variable in the LPS group, with lactate in some fetuses decreasing. Histologically, there was a reduction in reactive microglia ($P<0.05$) and caspase-3 +ve cells ($p<0.001$).

These data show that prolonged exposure to LPS reduces neural injury and this was associated with a rapid and better cardiovascular adaptation, particularly of BP and CaBF. However the lower FHR and lactates are of concern. Research shows that that lactate production is linked to adrenaline stimulation of the Na+/K+ ATPase pump, and that systemic lactate deprivation is detrimental to myocardial energetics, longer-term cardiovascular performance and outcome. This raises the question of whether LPS-treated animals could tolerate longer periods of asphyxia.

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ANTENATAL ALLOPURINOL PROTECTS THE FETAL HEART AND BRAIN AFTER ACUTE BIRTH ASPHYXIA IN LATE GESTATION FETAL SHEEP

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Aim: Free radical induced reperfusion injury is a recognized cause of brain tissue damage after birth asphyxia[1]. The xanthine-oxidase inhibitor allopurinol reduces the formation of free radicals and crosses the placenta easily[2,3]. Therefore, allopurinol is a promising therapeutic candidate to prevent tissue damage even before birth. We demonstrated earlier a cardioprotective effect of allopurinol during birth asphyxia in late gestation fetal sheep[4]. In the present study we tested the hypothesis that maternal treatment with allopurinol during fetal asphyxia would also limit the amount of ischemia-reperfusion (I/R) damage to the fetal sheep brain.
Methods: I/R was produced by repeated compressions of the umbilical cord (UCC, 5x10 min) in 10 chronically-instrumented fetal sheep at 0.8 of gestation. During UCC, either maternal i.v. allopurinol (20 mg/kg, I/R allo, n=5) or vehicle (I/R vehicle, n=5) was administered. Fetal brains were perfusion fixed 72h after I/R in these two groups and in a further 6 uninstrumented gestation-matched fetuses, which served as controls. Neuronal damage was determined using acid fuchsin/thionin staining under light microscopy. The proportion of neuronal damage was scored using a 6-point scale: 0 = no dead neurons, 1 = > 0-10%, 2 = > 10-50%, 3 = > 50-90%, 4 = > 90 - < 100%, 5 = 100% dead neurons[5].

Results: Relative to controls, I/R induced significantly greater neuronal damage in the hippocampal cornu ammonis zone 3 and 4 ($P < 0.05$, RM ANOVA + Bonferroni test). Maternal treatment with allopurinol during I/R restored neuronal damage towards control scores. Differences between groups were also prominent, although outside statistical significance, in the dentate gyrus and thalamus regions ($P=0.08$, RM ANOVA + Bonferroni test).
Conclusion: Besides cardiovascular benefits, maternal treatment with allopurinol offers potential neuroprotection to the fetal brain in the clinical management of perinatal asphyxia.

(1) Fellman et al. Pediatr Res 1997;41:599-606
(2) Palmer et al. Pediatr Res 1990;27:332-6
(3) Torrance et al. Pediatrics 2009;124:350-7
(4) Derks et al. Pediatr Res 2010;68:374-80
(5) Fraser et al. Dev Brain Res 2005;154:45-55

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DOES BIRTH ASPHYXIA IN THE SPINY MOUSE LEAD TO NEONATAL RENAL FAILURE?

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Background: Neonatal renal failure (NRF) has long been a major clinical complication following birth asphyxia. Clinical observations of renal function following birth asphyxia have been measured using urine and plasma for biomarkers of kidney failure. However, lack of an appropriate animal model means that very little is understood about the molecular and structural changes elicited in the kidneys, after an asphyxic insult at birth.
The Model: The spiny mouse is a precocial rodent species, such that its organ maturity at birth is comparable to human newborns. Of particular relevance for this study is the completion of nephrogenesis before birth, making the study of renal hemodynamics after birth asphyxia in this species particularly relevant to the human situation.
Method: On day 38 (term is day 39), pups were delivered by caesarean section or subjected to 7.5-8min of intrauterine asphyxia, after which the fetuses were expelled, resuscitated and cross-fostered to a lactating dam. After 24h, kidneys were collected and fixed for histological analysis or frozen for molecular analysis. Urine and plasma were collected for biochemical analysis. Kidney sections were stained with H&E and assessed for structural changes using an arbitrary scoring system.
Results: We have identified gross structural changes within the kidney in offspring, 24h after birth asphyxia. A reduction in overall kidney size and a decrease in the cortex to medulla ratio have been observed, along with localized reductions in tissue integrity. Specifically, we see shrunken glomerular tufts and disorganization of glomeruli within the cortex. There is evidence of necrosis and granular casting in the renal tubules within the medulla.
Discussion: These results indicate that kidney damage occurs in spiny mouse neonates after birth asphyxia. Ongoing studies are examining the functional consequences of these structural deficits and will determine whether the spiny mouse represents a model of birth asphyxia induced NRF.

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MATERNAL INFLUENCES ON PLACENTAL EPIGENETIC SIGNATURES IN PREGNANCIES OF OVERWEIGHT AND OBESE WOMEN

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Childhood obesity has reached epidemic proportions world-wide[1]. A major risk factor for childhood obesity is maternal overweight and obesity, also increasingly common[2]. This association is likely to reflect a combination of genetic and factors, including 'programming' of offspring by the intra-uterine environment. Maternal factors associated with obesity acting on the placenta may also indirectly affect the latter, including modification of placental epigenetic state and function. Maternal BMI is predictive of peroxisome proliferator activated receptor gamma coactivator 1a (*PPARGC1a)* methylation in the cord of LGA newborns[3], but the impact on DNA methylation of the gene for this metabolic regulatory molecule in the placenta is unknown. We therefore examined the influence of maternal BMI and related characteristics (maternal blood glucose, triglycerides, insulin) from mid gestation to term, in overweight and obese women, on DNA methylation in the promoter of *PPARGC1a* and a DMR of *IGF2*, in the placenta, collected at delivery in a subset of the LIMIT RCT (n=15). DNA was extracted, bisulphite treated and subjected to pyrosequencing. Maternal BMI, plasma triglycerides or insulin were not associated with DNA methylation of either loci. Maternal blood glucose in mid- but not later in gestation, was positively associated with placental *PPARGC1a* methylation at 4 of 5 sites (r=0.73, p=0.007 to r=0.845, p=0.001). In contrast, no maternal measure was associated with methylation in the *IGF2* DMR. Maternal glycaemia in mid gestation influences methylation of *PPARGC1a* in the placenta in overweight or obese women. Increased methylation of this transcriptional co-activator that regulates mitochondrial biogenesis and function may impair glucose and lipid homeostasis and alter placental function, exacerbating obesity related changes to the intrauterine environment. This also suggests that maternal obesity can program placental epigenetic state via maternal glycaemia early in pregnancy.

(1) de Onis M et al. 2010 Am J Clin Nutr 92: 1257
(2) Dodd JM et al. 2011 Maternal ANZJOG In press
(3) Gemma C et al. 2009 Obesity 17: 1032

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CORRELATING HISTOLOGICAL DAMAGE WITH NEURORADIOLOGICAL IMAGING IN A TERM LAMB MODEL OF BIRTH ASPHYXIA.

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Background: Birth asphyxia describes a severe lack of oxygen during labour and is associated with adverse outcomes involving permanent neurological impairment, or, in extreme cases, death. This study aims to establish the correlation between neuroradiological imaging and brain histopathology in a novel lamb model of birth asphyxia.

Methods: Birth asphyxia was induced through umbilical cord occlusion (UCO) at caesarean section in term lambs (~140 days). UCO was ceased when mean blood pressure fell to 20mmHg and lambs were immediately delivered by caesarean section. Control lambs did not receive UCO but were also delivered by caesarean section. Following delivery, both groups of lambs were resuscitated using standard neonatal guidelines and maintained for 72 hours. Magnetic resonance imaging (MRI) was performed on a 3T Siemens Vario at 12 and 72 hours after delivery using standardised sedation. T1, T2, Diffusion Weighted Imaging and MR Spectroscopy (MRS) were performed. Lambs were sacrificed immediately after the second MRI/MRS and brains collected for histological analysis.

Results: UCO resulted in significant changes to arterial pH (6.9±0.03), SaO2 saturation (SaO2, 4.1 ±1.3%), base excess (-14.2±1.8mmol/L) and lactate (7.5±1.3mmol/L) at the end of the UCO, compared to results prior to UCO [pH (7.3±0.02), SaO2 (73.2±2.3%), base excess (-1.4±2.3mmol/L), and lactate (2.1±0.2mmol/L)]. MRI in UCO lambs demonstrated a range of outcomes from no injury through to gross oedema, restricted diffusion, elevated lactate and reduced N-acetyl aspartate in the basal ganglia and thalami. The severity of these changes increases on the delayed scan. Histological analysis is being undertaken to determine pathology and cell death in susceptible brain regions.

Conclusion: Our model demonstrates that following birth asphyxia in lambs MRI/MRS changes are similar to those seen in human neonates and provides a model to determine the progression and location of brain injury.

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CARDIOMYOCYTE GROWTH AND MATURATION DURING MID TO LATE GESTATION AND THE EFFECT OF PRETERM BIRTH

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Background: Preterm birth affects 8-12% of all pregnancies and is the leading cause of neonatal morbidity and mortality. We have previously shown that moderate preterm birth in lambs results in cardiomyocyte hypertrophy, derangements of cardiomyocyte maturation and increased interstitial collagen deposition. Therefore the aim of this study was to determine whether we would observe similar effects in humans born preterm.

Method: Archived heart tissue obtained at perinatal and neonatal autopsy was analysed. We established the normal growth and maturation parameters of human cardiomyocytes using gestational controls (*in utero* demise) (n=37) and comparing these hearts to neonates who died following preterm birth (n=30). We used confocal microscopy to examine cardiomyocyte volume, ploidy and maturation. Immunohistochemistry was used to analyse cardiac progenitor cell population and cardiomyocyte proliferation. Image analysis of picrosirius red-stained sections was used to quantify collagen deposition.

Results: Cardiomyocyte growth and maturation was unaffected by preterm birth, continuing along the normal developmental trajectory. A near cessation of cardiomyocyte proliferation occurred after preterm birth. This was coupled with a reduction in the number of cardiac progenitor cells (c-kit+/Lin-).

Conclusions: There was no effect of preterm birth on cardiomyocyte size, maturation or ploidy. However, we have demonstrated that within 12 hours of preterm birth, the rate of cardiomyocyte proliferation is reduced. This may lead to a reduction in cardiomyocyte endowment later in life. The reduced number of cardiac progenitor cells and cessation of cardiomyocyte proliferation are of crucial importance because this may predispose those born preterm to developing cardiovascular disease later in life.

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LUNG REPAIR AFTER VENTILATOR-INDUCED INJURY OF THE EXTREMELY PRETERM OVINE LUNG

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Introduction: Mechanical ventilation (MV) of preterm infants can cause lung injury, which is associated with bronchopulmonary dysplasia (BPD). However, only ~1/3 of preterm infants develop BPD. We have previously shown that lung injury in very preterm lambs (~26 weeks human gestation) can resolve spontaneously within 15 days. However, it is not known if the extremely preterm lung (~23 weeks human gestation) is capable of repair following MV-induced injury. Aim: To determine if the extremely immature lung has the capacity to repair following injury induced by brief MV. Methods: Under aseptic conditions, the head and chest of fetal sheep at 110d GA (gestational age, term=147d GA) were exposed and fetuses ventilated for 2h. Fetuses remained in utero until tissue collection at 24h and 15d after MV (n=6-7/group). Unventilated, age matched fetuses were used as controls. Lungs were processed for image analysis and qRT-PCR. Results: At 24h after MV the lungs displayed signs of injury, including, haemorrhage, non-uniform inflation and abnormal elastin deposition. The area of lung occupied by tissue increased by 15% ($p<0.05$) and secondary septal crest density decreased by 22% ($p<0.05$). mRNA levels of the repair genes metallothionein and urokinase receptor were 2.7+0.4 and 2.9+0.8 fold higher than in unventilated controls ($p<0.05$). Early response gene and inflammatory cytokine mRNA levels were normal at 24h after brief MV. Fifteen days after MV there were no differences in lung structure between MV and control fetuses. Elastin deposition was restored and predominantly located in the wall and tips of developing alveoli. Conclusion: The extremely immature lung can undergo spontaneous repair after brief, MV induced injury and this may be mediated by increases in the repair genes metallothionein and urokinase receptor. Identifying the mechanisms that lead to spontaneous lung repair may lead to novel therapeutic treatments for preterm infants at risk of developing BPD.

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LACTOFERRIN: A NECESSARY INCLUSION FOR PREGNANCY AND NEWBORN SUPPLEMENTS

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The maternal diet is critical for optimal fetal and neonatal growth and development. During pregnancy, supplements are often used to ensure women meet their elevated recommended daily intake of nutrients (folic acid and other B-group vitamins, iron, iodine, omega-3 fatty acids). Despite the awareness of supplements during pregnancy, nutrition-related morbidity is still very prevalent. Iron deficiency is a concern amongst pregnant women as the administration of iron supplements often fail to raise iron status due to the poor bioavailability. Another concern revolves around the development of the neonatal immune system. Allergies are an increasing concern in industrialized countries and infections are the number one cause of easily preventable deaths of newborns in developing countries. A solution to these problems may lie with the bioactive protein, lactoferrin. The only food source of lactoferrin is mammalian milk, however cow's milk only contains trace amounts. The primary role of lactoferrin is to boost the innate immune system with its potent microbistatic and microbicidal properties. It has a very strong binding affinity to iron and is able to significantly increase iron and haemoglobin levels in pregnant women more than 500 times greater than ferrous sulphate supplements. Lactoferrin supplements have shown to decrease the risk of sepsis and the severity and duration of illness. We have used patent technology to design a supplement that can be administered to pregnant women and neonates. The formula containing lactoferrin, omega-3, iron, iodine, B-group vitamins and other nutrients can substantially increase iron status in pregnant women and may reduce the incidence of allergies and infections in newborns while optimizing brain development. We have established a clinical trial protocol to determine if our nutritional supplement can significantly reduce the incidence and severity of illness in newborns and improve iron status in pregnant women.

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THE EFFECT OF MECHANICAL VENTILATION AND ANAESTHESIA ON THE PRETERM OVINE BRAIN.

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Background: The occurrence of germinal matrix-intraventricular haemorrhage (GM-IVH) is common in very preterm infants and potentially contributes to permanent brain injury. Many very preterm neonates are exposed to anaesthetic agents and require mechanical ventilation after birth. These interventions could also contribute to brain injury. The aim of this study was to assess the effect of mechanical ventilation and anaesthesia on the preterm ovine brain. Methods: Ewes were anaesthetised and preterm lambs (n=4, 127 days gestational age (GA); term is 147 days) were intubated and ventilation was initiated with a positive end-expiratory pressure (PEEP) of 4 cmH2O. At 30 min, PEEP was randomised to 6, 8 or 10 cmH2O with a 10 min period at 4 cmH2O between each PEEP increase. After 2.5 h lambs were killed and the brain collected. Control twins (n=4) were used as anaesthetised unventilated controls (AUVC). An additional group of lambs (n=4) was included that were matched for GA but not exposed to anaesthetic (UVC). Immunohistochemistry was used to assess vascular extravasation (albumin), and activated microglia/marcophages (lectin). Gross histopathological changes were assessed on H&E stained sections. Results: The brains of ventilated lambs had increased albumin and lectin staining in both periventricular and subcortical white matter. Greater incidences of parenchymal abnormalities were seen predominantly in white matter of these lambs. AUVC lambs had increased lectin staining relative to non-anaesthetised UVC. Conclusions: These preliminary findings suggest that 2.5 h of mechanical ventilation results in profound inflammatory responses and evidence of blood-brain barrier compromise, which are associated with gross anatomical abnormalities in the white matter. Brief exposure to anaesthesia appears to also enhance the inflammatory response in white matter however whether this is protective or damaging the preterm brain remains to be elucidated.

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CHRONIC IN UTERO EXPOSURE TO LOW-DOSE ENDOTOXIN IMPAIRS EEG MATURATION

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Background: Neurodevelopmental disability is a common sequelae of premature birth, and has been strongly linked with chorioamnionitis. Although acute sepsis or exposure to high-dose LPS have been associated with impaired brain growth and maturation it is unclear whether sub-clinical infection is also associated with impaired neurodevelopment. We examined the hypothesis that chronic, low-dose LPS over 5 days would increase pro-inflammatory cytokines and cortisol as a measure of fetal stress, and impair EEG maturation in preterm fetal sheep. Methods: Chronically instrumented 103 day old (0.7 gestation age: term 147 days) fetal sheep in utero were randomized into 2 groups: saline infusion (n=9) or LPS infusion (n=6). Fetuses received either LPS as a continuous low dose infusion (100 µg over 24h, followed by 250 µg/24h for 4 days), or the same volume of normal saline. Arterial blood samples were taken for cortisol and cytokine analysis. The proportion of power in the EEG spectrum was calculated for delta (0-4 Hz), theta (4-8 Hz), alpha (8-13 Hz) and beta (13-22 Hz) frequencies. Results: There were no significant differences in EEG waveforms during LPS infusion. After LPS infusion alpha activity was lower in the LPS group from day 6, beta activity was lower from day 7, delta activity higher from day 8, and theta activity lower from day 9 compared to the saline group ($p<0.05$). Cortisol and IL-6 were significantly higher after the start of LPS infusion compared to baseline ($p<0.05$). Conclusion: Low-dose endotoxin exposure is associated with evidence of EEG dysmaturation with mild elevations of plasma cortisol and pro-inflammatory cytokines in preterm fetal sheep.

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H2S; A NOVEL PLAYER IN THE TRANSITIONAL MICROCIRCULATION OF PRETERM NEONATES?

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Background: Endogenously produced hydrogen sulphide (H2S) is a known vasodilator and has been implicated in microvascular function and dysfunction in some disease states in the adult circulation. Nitric oxide and carbon monoxide, the other identified gasotransmitters, have previously been shown to play crucial roles in the transitional circulation of the preterm neonate. It was therefore hypothesised that H2S may also contribute to the significant microvascular dysfunction and circulatory compromise seen in very preterm neonates during this critical period.
Methods: H2S is present in very low concentrations within the microcirculation and is cleared by exhalation or converted to a stable metabolite, thiosulphate, which is excreted in urine. A modification of the Sorbo spectrophotometric method was validated for the measurement of thiosulphate in newborn neonatal urine samples as a marker of total body turnover of H2S. Results were tested by unpaired t-tests between groups and paired t-tests between days of postnatal life. All values are mean±SEM.
Results: Results show that this method is sensitive enough to detect low levels of thiosulphate in samples collected from both term and preterm infants in early extrauterine life. On day 1 of postnatal life, thiosulphate was significantly higher in very preterm neonates 0.32±0.03μmol/ml (n=13; ≤28 weeks completed gestation) than preterm neonates born at later gestational ages 0.21±0.03(n=11; p=0.022). Levels remained stable in both groups on day 2 of life.
Conclusions: This method is suitable for the assessment of urinary thiosulphate in the newborn neonate. The results provide the first evidence that H2S may play a significant role in the control of microvascular tone in the transitional circulation of the neonate. Further investigation to verify this is underway and this opens new therapeutic possibilities in this vulnerable group.

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MARKERS OF LUNG INJURY IN MECHANICALLY VENTILATED PRETERM SHEEP EXPOSED TO INTRAUTERINE INFLAMMATION.

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Background: Ventilation-induced lung injury (VILI) in preterm infants may contribute to bronchopulmonary dysplasia (BPD). Intrauterine inflammation is often present before preterm birth and may exacerbate VILI and increase the risk of BPD. The early response genes, cysteine rich-61 (CYR61) and connective tissue growth factor (CTGF) are early markers of VILI.

Aim: To determine if intrauterine inflammation exacerbates ventilation-induced increases in CYR61 and CTGF expression.

Methods: At ~118 days of gestation (d; term ~147d), pregnant ewes received an intra-amniotic injection of lipopolysaccharide (LPS; E coli 055:B5; 20mg) or saline. At ~125d lambs were either killed (LPS, n=5; control, n=6) or delivered and mechanically ventilated for 40 minutes (LPS, n=5; control, n=4). Lung CTGF and CYR61 mRNA levels were measured using Real-Time PCR and compared using 2-way ANOVA.

Results: Ventilation increased CYR61 mRNA levels (p=0.0003) in saline (unventilated 1.0 ± 0.3 vs ventilated 367.3 ± 137.1) and LPS lambs (unventilated 0.6 ± 0.2 vs ventilated 699.3 ± 207.5). CTGF mRNA levels in saline (unventilated, 1.0 ± 0.3 vs ventilated 18.2 ± 4.7) and LPS lambs (unventilated, 0.4 ± 0.1 vs ventilated, 48.4 ± 27.5) were increased by ventilation (p=0.048). CYR61 and CTGF mRNA levels were not different between (ventilated or unventilated) saline and LPS lambs.

In ventilated LPS lambs, IL-1β and IL-8 mRNA levels were ~50% lower, whereas the IL-6 mRNA level was ~60% higher. Data are expressed relative to ventilated saline lambs. These effects were not statistically significant.

Conclusions: Prior exposure to intrauterine inflammation does not alter the ventilation-induced increases in early markers of lung injury, CYR61 and CTGF. These data suggest that intrauterine inflammation neither exacerbates nor protects the immature lung from ventilator-induced lung injury after birth.

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VITAMIN D DEFICIENCY, COMMENCING IN UTERO, AFFECTS CARDIAC FUNCTION AND INCREASES THE SUSCEPTIBILITY TO MYOCARDIAL ISCHEMIA/REPERFUSION INJURY IN ADULTHOOD

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Background: Vitamin D deficiency is an emerging health problem. Some ethnic/religious groups are exposed to life-long vitamin D deficiency, beginning in utero.

Aims: To investigate the effect of life-long vitamin D deficiency in adult rats on myocardial capillarisation, cardiac function and the susceptibility to ischemia/reperfusion injury.

Methods: Four week old Sprague-Dawley female rats were fed either a vitamin D deplete or vitamin D replete (control) diet for 6 weeks prior to pregnancy, during pregnancy and throughout lactation. Offspring remained on their respective diets until adulthood. Hearts of 16 week old vitamin D deficient and control rats (n = 8/group) were mounted on a Langendorff apparatus and cardiac function and the response to ischemia/reperfusion assessed. In separate cohorts myocardial capillarisation was quantified using stereological techniques.

Results: Basal and stimulated heart function was not altered, although coronary flow was significantly reduced (p = 0.007) in vitamin D deficient rats. Strikingly, infarct area was 2-fold greater in vitamin D deficient hearts of both males and females (p = 0.006 & p = 0.03, respectively). Myocardial vascularisation was not different between the groups.

Conclusion: Hearts of vitamin D deficient rats were particularly susceptible to ischemia/reperfusion injury. Dysregulation of coronary flow is likely to be contributing to the increased susceptibility of ischemia/reperfusion injury, but this is not attributed to myocardial vascularisation.

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RHO-KINASE INHIBITION MODIFIES PULMONARY VASCULAR REACTIVITY IN CHRONICALLY HYPOXIC NEONATAL LAMBS IN THE ANDEAN *ALTIPLANO*.

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Background: Neonatal pulmonary hypertension is a frequent pathology found in pregnancies under chronic hypoxia1. Rho-kinase (ROCK) is an enzyme that promotes vasoconstriction and has an important role in the regulation of pulmonary vascular tone2. Our objective was to examine the cardiopulmonary effects of a chronic treatment with fasudil, a ROCK inhibitor, during early neonatal life in pulmonary hypertensive lambs. Methods: 10 lambs gestated at Putre, Andean *altiplano* (3,600m) were used for this study. Lambs were catheterized at 3 days old and they were randomly divided in 2 groups receiving either saline (Control) or fasudil (3mg.kg-1.d-1, Fas) during 7 days. At 15 days old, the neonates were submitted to an acute hypoxic protocol (1h air, 1h 10% O2, 1h air) and their cardiopulmonary response was registered. In addition, vascular reactivity (wire myography) and morphology (histology) was assessed in small resistance pulmonary arteries. Results: Fas decreased pulmonary pressure the first 3 days of treatment with no changes in systemic circulation. During the acute hypoxic challenge Fas markedly diminished the pulmonary pressure (Fas: 30.3 ± 0.4 *vs.* Control: 41.8 ± 0.4 mmHg) and resistance. Small pulmonary resistance arteries from Fas neonates showed similar contractility (K+ response) relative to Control. However, Fas markedly decreased the maximal response to a thromboxane mimetic, relative to Control (Fas: 112 ± 5 *vs.* Control: 148 ± 5 %Kmax). Finally, small pulmonary arteries from the Fas group have less muscular area than Control (Fas: 30.0 ± 1.4 *vs.* Control: 47.4 ± 3.7 %). Conclusion: We show, for the first time in high altitudes, that a ROCK inhibitor improves pulmonary circulation function and reverts the vascular remodeling induced by chronic hypoxia in newborn lambs. This can be a potential alternative treatment in neonates that underwent chronic hypoxic conditions, for instance in high altitude populations.
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(1) Abman SH. Recent advances in the pathogenesis and treatment of persistent pulmonary hypertension of the newborn. Neonatology 91: 283, 2007.

(2) Badejo et al. Analysis of pulmonary vasodilator responses to the Rho-kinase inhibitor fasudil in the anesthetized rat. Am J Physiol 295: L828, 2008.

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A COMPUTER SIMULATION SHOWED THAT AMNIOTIC FLUID PRESSURE IN THE THIRD TRIMESTER WOULD BE KEPT STABLE AGAIST UTERINE TONUS BY AUTOMATIC CHANGE OF ITS VOLUME.

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Introduction: It is well-known that amniotic fluid volume (AFV) declines in the third trimester of pregnancy. However, the cause and the role of this decline are uncertain. We thus explored them from a physical point of view.

Methods: Fetal body weight (FBW), AFV and amniotic fluid pressure (AFP) in a standard case of 40 weeks is 3258g, 360ml and 10mmHg respectively. We simplified the fetal figure as follows: the head was an ellipsoidal sphere having 4.5x5x7 cm of radius, the trunk was an ellipsoidal cylinder having 5x5.3 cm of radius with 15 cm of length and the pelvis was a half ellipsoidal sphere having 6x10x10 cm of radius. Amniotic membrane enveloped this model of fetus and contained amniotic fluid in space between the fetus and the membrane. We then assumed that the amniotic space would grow or shrink according to gap between AFP and uterine muscle tension (UMT) and that amniotic membrane constituted a circular curve having a radius of R between fetal head and pelvic region. We applied a physical theory to this model and obtained a relation of AFP=UMT/R.

Results: AFP was 10mmHg and AFV was 360ml (R=40.6cm) in the standard case. Thus UMT would be 406mmHg x cm. If UMT doubled (813mmHg x cm) and continued, our calculation showed that AFP was 20mmHg initially, however as AFV gradually decreased to 285ml (R=80.9cm), AFP declined to 10.04mmHg. On the other hand, if UMT shifted to a half of the standard (203mmHg x cm), our calculation showed that AFP was 5mmHg initially, however as AFV gradually increased to 700ml (R=20.2cm), AFP returned to 10.05mmHg.

Conclusions: Our computerized model showed amniotic fluid volume would shift automatically according to uterine tonus change. The volume shift would have an important role in stabilizing amniotic fluid pressure.

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EFFECTS OF PRETERM BIRTH ON THE MATURATION OF AUTONOMIC CONTROL DURING SLEEP IN INFANCY

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Objective: In full term infants, maturation of the autonomic nervous system occurs within the first 6 months of life, with parasympathetic input to the heart increasing with age. Preterm infants have been found to exhibit deficits in autonomic cardiovascular control at term-equivalent age, which may underpin their increased risk for Sudden Infant Death Syndrome (SIDS). However, little is known about autonomic activity during sleep beyond term-equivalent age in preterm infants. We aimed to examine the effects of age and preterm birth on heart rate variability (HRV) as a measure of autonomic control during sleep in infants within the first 6 months of corrected age (CA).

Methods: Preterm (n=25) and term (n=20) infants were studied longitudinally at 2-4 weeks, 2-3 months and 5-6 months CA using daytime polysomnography during quiet (QS) and active (AS) sleep. Autonomic control was assessed from spectral indices of HRV. Low frequency (LF, reflecting sympathetic+parasympathetic activity), high frequency (HF, reflecting parasympathetic activity), the LF:HF ratio (reflecting sympathovagal balance) and Total power (total variability) spectral indices were calculated.

Results: In the preterm group with advancing age, HF power increased during QS ($p<0.05$) and AS ($p<0.05$) and Total power increased during QS ($p<0.05$). In contrast the LH/HF ratio decreased with advancing age in preterm infants during AS ($p<0.05$). Compared to term infants, at 5-6 months preterm infants had a lower LF power ($p<0.05$), HF power ($p<0.05$) and Total power ($p<0.05$).

Conclusions: Similar to term infants, preterm infants displayed an increase in paraympathetic activity and decrease in sympathetic activity with age. However by 5-6 months CA, preterm infants exhibited lower HRV indices, indicative of a global depression of autonomic control at this age. Depressed heart rate control in preterm infants could increase the vulnerability to cardiovascular instability during sleep and may play a role in the mechanism of SIDS.

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NEUROSTEROID REPLACEMENT THERAPY IN THE PRETERM NEONATE.

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Premature birth is associated with poor neurodevelopmental outcomes. The neurosteroid allopregnanolone (AP) is metabolised from progesterone via 5α-reductase (5αR) action. AP modulates neural excitability and is neuroprotective. Concentrations of AP and progesterone are high during gestation but fall rapidly following delivery. This study aims to examine the neurodevelopmental role of AP in a preterm neonatal guinea pig model.

Guinea pig neonates were delivered by c-section at 62-63 days gestation (preterm) or at 69 days (term=70 day) following betamethasone administration 24hrs before delivery. Neonates received CPAP, surfactant, assisted feeding and were housed in an incubator. At PND1 (postnatal day 1; term & preterm) and PND8 (term equivalent preterm) brains were processed for allopregnanolone radioimmunoassay, 5αR immunoblotting, and MBP (Myelin Basic Protein) and GFAP (Glial Fibrillary Acidic Protein) immunohistochemistry. In order to assess progesterone therapy, PND1 preterm neonates received progesterone (16mg/kg) or vehicle (2-hydroxypropyl-cyclodextrin) injections at 1 and 6hrs. Statistical analysis was by1-way ANOVA.

Term and preterm neonates exhibited a significant decrease in brain allopregnanolone concentrations after birth. At PND1, preterm neonates demonstrated reduced expression of MBP, GFAP and 5αR2 when compared to term PND1 levels ($p<0.05$). At PND8 in preterm animals, MBP expression in the CA1 region remained significantly reduced. Progesterone treatment successfully increased salivary progesterone and brain allopregnanolone concentrations when compared to vehicle controls ($p<0.05$).

We have established a model of neonatal prematurity in the guinea pig. At term equivalent age late developmental processes in these preterm neonates have not caught up to term levels. Short-term progesterone therapy successfully increases brain concentrations of allopregnanolone, suggesting that the immature neurosteroid system in the preterm brain is sufficient for allopregnanolone synthesis if precursors are made available. These findings support the investigation of progesterone replacement in preterm neonates as a possible therapeutic avenue to improve brain development and neurological outcomes.

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FOLLISTATIN AND THE TREATMENT OF HYPEROXIA-INDUCED LUNG INJURY IN NEONATAL MICE

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Background: Bronchopulmonary dysplasia (BPD) is a common sequela of very preterm birth and is associated with significant long term respiratory and neurological impairments. There is no current treatment for BPD. Activin A - a TGF-β family member – has been shown to play an important role in various inflammatory diseases, including lung inflammation, fibrosis and scarring. During lung injury, activin A drives fibroblast differentiation into myofibroblasts, leading to the deposition of elastin and collagen and thereby causing fibrosis and disruption of lung architecture. In adult models of lung injury, modulation of activin by administration of its binding protein follistatin can prevent acute inflammation and subsequent scarring. Whether follistatin can prevent or repair BPD has not been explored. *Aims:* To evaluate the effect of follistatin on hyperoxia-induced neonatal lung injury. *Methods:* We used a hyperoxia mouse model of BPD. Newborn mice were placed in a hyperoxia (85% 02) chamber from birth and litters then randomised to receive either subcutaneous saline or follistatin (25 or 100 μg/kg/day groups) from day 5-13. Animals were culled at day 14 for the assessment of lung structure (mean linear intercept, secondary septal crest density, interstitial thickness), fibrosis (collagen, elastin, α- smooth muscle actin), inflammation (total white cell count, neutrophils, macrophages) and activin signalling (activin and TGF- β, SMADs). *Results:* Preliminary results show that in hyperoxia pups, compared to saline treated controls, follistatin administration at 25μg/kg/day reduces pulmonary white cell infiltration and interstitial thickness. However they also show a reduction in weight gain in follistatin treated groups compared to controls. Completion of analyses is underway. *Conclusion:* These results suggest that follistatin may mitigate neonatal lung inflammation and scarring induced by exposure to hyperoxia. These pilot data would support further assessment of follistatin as a therapy in preterm neonates, however further assessment of the positive and negative effects of follistatin is required.

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COULD THE NEWBORN HEART BE RESISTANT TO HYPOXIC INJURY?

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Background: Using a model of birth asphyxia in the precocial spiny mouse, we have previously shown that a maternal diet supplemented with creatine from mid-pregnancy improves offspring survival and protects the newborn brain, diaphragm and skeletal muscle from asphyxia-induced damage. The aim of this study was to assess the effect of asphyxia on the newborn heart, and if injured, whether maternal creatine supplementation offered protection.

Method: Pregnant spiny mice were fed a control or 5% creatine-supplemented diet from day 20 of gestation to term (day 39). On day 38, pups were delivered by caesarian section, or subjected to 7.5min of asphyxia. Surviving neonates were cross-fostered to nursing dams for 24h. At post-mortem hearts were fixed in buffered formalin for histology or frozen for later RNA extraction. The mRNA expression of genes known to be responsive to hypoxia (Hif1α), promote apoptosis (Bax), drive cardiomyocyte development (Igf1, Igf1r, creatine transporter), as well as genes involved in muscle atrophy (Atrogin-1, MuRF-1 and Myostatin) were examined by qPCR. Transverse sections of the heart were stained with H&E to assess the gross structure, and measurements of ventricular wall thickness were determined using Image J.

Results: Heart weight was not different between groups. There were no significant differences in the mRNA expression of any genes of interest in the 24h old newborn heart between groups. The gross structure of the heart was not different between groups and there were no differences in wall thickness or the ratio of wall thickness to heart or body weight, 24h after asphyxia.

Conclusions: In contrast to the severe damage to skeletal and diaphragm muscle of 24h old pups after birth asphyxia, no obvious structural damage to the heart was observed. Studies of heart function are ongoing.

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OXIDATIVE STRESS INDUCED UNFOLDED PROTEIN RESPONSE IN PREECLAMPSIA

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Background: Profound oxidative stress originating from ischaemic-repefusion injury following inadequate spiral artery transformation is common in preeclampsia (PE). Accumulation of misfolded immature proteins in the ER following oxidative stress provokes the unfolded protein response (UPR). The role of UPR in the pathophysiology of PE remains largely unexplored. Aim: To determine the presence of UPR markers in PE placentae, as well as determine the ability of activin A to directly mediate UPR in vitro. Hypothesis: UPR seen in PE is mediated by a stressor such as activin A, which reduces Nrf2-mediated transcription of antioxidant genes. This will translate to elevated levels of markers of UPR in PE placentae. Methods: Protein expression was analyzed by western blot and/or by immunohistochemistry. XBP1-mRNA splicing was performed using RT-PCR. Nrf2 regulated antioxidant genes were analyzed using qRT-PCR. Results: Levels of CHOP in placental lysates were approximately 25% higher in PE compared to NP (**$p<0.001$). The proportion of pPERK to total PERK was 4.9-fold greater in PE placentae compare to healthy placentae ($p<0.001$). Treatment of HUVECs with activin A caused XBP1 splicing, indicative of UPR. Activin A treatment of HUVECs delayed nuclear translocation of Nrf2 which was restored by apocynin or FS288. Treatment with activin A increased expression of Nrf2 target antioxidant gene, HO-1 ($p<0.05$), whilst not activating other Nrf2 target antioxidant genes. The expression of GPX1 ($p<0.05$) was increased following co-culture of activin A treated HUVECs with FS288, whereas expression of SOD3 ($p<0.01$) and NQO1 was increased. Conclusion: Activin A can directly induce the UPR in HUVECs. We also provide evidence of increased UPR in PE, which we believe is due to reduced Nrf2 signaling. Our findings suggest a protective role for Nrf2 in pregnancies affected by PE and/or IUGR. The targeting of the Nrf2-ARE pathway would be a novel approach to identifying candidate therapeutics.

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ROLE OF REGULATORY T CELLS S IN HUMAN AMNION EPITHELIAL CELL MEDIATED LUNG REPAIR

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Background: Idiopathic pulmonary fibrosis (IPF) is an incurable chronic disease with high levels of morbidity and mortality. Regenerative medicine using human amnion epithelial cells (hAECs) has become a popular approach in modulating repair in lung diseases. hAECs, derived from the placenta, have been shown to have potent anti-inflammatory and antifibrotic effects. CD4+CD25+Foxp3+ regulatory T cells (Tregs) are also involved in the modulation of fibrotic lung disease. It is hypothesised that hAECs require an adaptive immune system to exert their anti-inflammatory and anti-fibrotic effects. We also hypothesised that the adoptive transfer of Tregs will facilitate the modulation of repair following hAEC administration. Aims: Determine the effects of hAECs administration in an immunocompromised mouse model of lung disease and demonstrate that Tregs are required for hAEC-mediated repair. Methods: Bleomycin (6U/mL) was instilled intranasally to induce lung disease in Rag1-/- mice prior to an adoptive transfer of 0.5 million Foxp3-GFP positive lymphocytes intravenously. Four million hAECs were administered by intraperitoneal injection, 24 hrs post-bleomycin challenge. At day 7, inflammatory gene expression was examined and extent of fibrosis, collagen content and α-SMA expression was investigated at day 14. Results: Gene expression for IL-6, TGF-β, IFN-γ and TNF-α increased after bleomycin insult and was decreased only in mice receiving Tregs (*$p<0.05$). Ashcroft score of fibrosis and inflammation, collagen deposition and α-SMA levels increased after bleomycin administration and was reduced only in mice receiving Tregs (*$p<0.05$). Conclusion: The interaction of Tregs with hAECs is important for the anti-inflammatory and anti-fibrotic effect of hAEC therapy.

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ROLE OF MACROPHAGES IN HUMAN AMNION EPITHELIAL CELL MEDIATED LUNG REPAIR

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Background: We previously reported on the ability of placental derived stem cells and stem-like cells to mediate repair in the bleomycin model of lung injury. We hypothesized that one mechanism by which hAECs mediate repair is through the influence of macrophage polarisation, specifically by directing macrophages from the pro-inflammatory M1 phenotype to the M2 phenotype responsible for wound healing. Methods: 6 to 8-week old female C57Bl6 mice were administered a single intranasal dose of bleomycin (8U/kg) and 4 million hAECs were administered via intraperitoneal injection. Animals were culled 8hrs, 2, 3, 5 or 7 days following hAEC administration. Bronchoalveolar lavage fluid was collected, as were lung tissues, for delineation between alveolar and interstitial macrophages. Macrophage number was determined by FACS sorting for CD45+ F4/80+ cells, and polarisation was determined by CD86 and CD206 surface markers. Polarity was confirmed by qPCR for arginase-1, FIZZ-1, Ym1/2 and NOS-2 gene expression. Results: We observed a significant influx of macrophages into the lungs 8 hours following hAEC administration ($p<0.05$), however, total macrophage infiltrate equalised between treatment groups by day 3. Polarisation of macrophages became apparent by day 7 ($p<0.01$) where macrophages were largely M2 in animals administered hAECs while bleomcyin injured animals only administered vehicle control were predominantly M1 ($p<0.01$). Conclusion: This is the first study to document the effect of hAECs on the polarisation of macrophages. Since the amnion is able to strongly express IL-4 and IL-14, which stimulate polarisation of macrophages to the M2 phenotype, we believe that this a potential mechanism by which hAECs can influence the cycle of scarring and wound healing.

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EFFECTS OF FETAL SEX ON PRORENIN PRODUCTION BY THE DECIDUA.

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Human intrauterine tissues contain the renin-angiotensin system (RAS). We have shown that in term decidua the expression of prorenin mRNA is higher than all other intrauterine tissues. Decidua was collected at elective caesarean section (37-40 weeks) and mRNA levels measured by real-time PCR. In pregnancies in which the fetus was female, decidual prorenin mRNA levels were significantly higher (P=0.004, n=9) than decidua from pregnancies in which the fetus was male (n=15).

We aimed to see if this sexual dimorphism persisted in decidual explants and to find out if decidua collected from pregnancies which carried a female fetus secreted more prorenin than decidua from women carrying a male fetus. Decidua (n=6; 3 female, 3 male) was dissected 1 cm from the placental margin, divided into 0.5 cm2 pieces and pre-incubated at 370C with 95% air and 5% CO2 for 24 h in DMEM/F-12 medium, then 4 pieces of decidua were placed in each well of a 6 well plate with 2 ml of incubation media. Prorenin mRNA abundance and secretion of prorenin (using an ELISA) into the media were measured.

48 h after removal from the uterus, prorenin mRNA abundance in decidua from ‘female' pregnancies was significantly greater than in decidua from ‘male' pregnancies (P<0.001). Prorenin levels, measured in media collected after the last 24 hr incubation were greater in ‘female' pregnancies (4.88 ng/ml ± 0.95) than from ‘male' pregnancies; media from the latter contained very little prorenin (0.13 ng/ml ± 0.06, P<0.01).

The RAS is a powerful system with a wide-range of actions. The striking difference in prorenin production between decidua collected from pregnancies with female babies compared with decidua from those with male babies may have far reaching consequences in understanding intrauterine growth and labour and could account for sex specific patterns of pregnancy complications.

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DO NEUROSTEROIDS PROTECT THE DEVELOPING BRAIN OF THE GROWTH RESTRICTED FETUS?

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Background: Intrauterine growth restriction (IUGR) is a severe complication of pregnancy and is associated with neuropathologies such as cerebral palsy. Neurosteroids synthesised de novo in the brain are deemed to protect against acute hypoxic insults. This study aims to examine whether neurosteroids are altered in the IUGR brain and to determine if there is a correlation between regional changes in brain neurosteroid levels and brain injury.

Method: Pregnant ewes underwent surgery at 110 days gestation. Single umbilical artery ligation (SUAL) was performed to induce IUGR. One group of animals (short-term IUGR) were kept for 7 days and exposed to betamethasone (or saline) on day 5 and 6 after surgery. The second group of long-term IUGR animals lambed naturally and were sacrificed 24 hours after birth. Brain and plasma were collected for determination of allopregnanolone (AP) levels.

Results: In the long-term IUGR animals, plasma AP levels were increased in fetal and newborn samples; this was significant at 125 and 130 GA (91.6 ± 9.7; 98.04 ± 10.3 pmol/ml) compared to controls (64.8 ± 8.3; 75.9 ± 7.5 pmol/ml; P < 0.05). In control animals, plasma AP concentration increased over gestation, peaking at 140 days. Within the brain of term control versus IUGR lambs, there was no difference in AP concentrations in the cortex (9.9 ± 1.1 and 10.4 ± 0.8 pmol/ml), striatum (10.9 ± 0.7 and 11.5 ± 1.0 pmol/ml), periventricular white matter (13.2 ± 2.1 and 8.9 ± 1.4 pmol/ml) and the cerebellum (8.8 ± 0.9 and 8.1 ± 0.9 pmol/ml). AP levels are currently being assessed in the short-term IUGR animals, with and without betamethasone.

Conclusions: Circulating levels of the neurosteroid allopregnanolone are elevated in late gestation IUGR fetuses, in response to long-term chronic hypoxia; this may protect the vulnerable IUGR brain.

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PREGNANCY, PLACENTATION AND FETAL GROWTH IN THE SPINY MOUSE

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Background : Optimal fetal growth is known to be vitally important for the later health of offspring, with appropriate growth of the fetus and its preparation for birth being a major role of the placenta. The species we use for our studies is the spiny mouse (*Acomys cahirinus*), a rodent species that gives birth to precocial offspring after a relatively long gestation (39 days). This means that the placenta, unlike that of other rodent species, must support fetal growth for an extended period of time, and provide adequate nutrition for the *complete* development of fetal organs before birth, as occurs in human pregnancy. The aim of this project is to describe the growth patterns of male and female fetuses and their placentas across gestation.

Method : Maternal, fetal and placental weights on each day of gestation from day 15 till term, was compiled to generate sex-specific fetal, maternal and placental growth curves. Fetal and placental tissues processed and subjected to histological analysis.

Results : Fetal spiny mice undergo exponential growth between day 23 and 37 of gestation. Male fetuses were heavier than females at day 23, but not at day 37. Placental weight increases uniformly and continuously until day 37. Within the placenta, the exchange region (labyrinth) can be identified from day 20; from this time the labyrinth increases the proportion of the placenta it occupies, and is larger than the junctional zone from day 28 until term. Histologically, at day 37 the labyrinth:junctional zone ratio was significantly greater for female vs. male fetuses.

Significance: These preliminary data suggests that, like the human, some differences exists between the growth patterns of the sexes *in utero*. These may be attributed to the differences in placental parameters observed within the study, but the origin of these differences (fetal? placental?) is not yet known.

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HISTOLOGIC CHORIOAMNIONITIS IS PRESENT IN APPROXIMATELY ONE-FOURTH OF PATIENTS WITH PRETERM LABOR AND INTACT MEMBRANES AND LOW AF WBC COUNTS AND IS A SIGNIFICANT RISK FACTOR FOR INTRA-AMNIOTIC INFLAMMATION AND RDS OF NEWBORNS

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OBJECTIVE : An elevated Amniotic fluid(AF) white blood cell(WBC) count is traditionally known to be a powerful predictor for positive AF culture, histologic chorioamnionitis(HCA) and early-onset neonatal sepsis(EONS) among preterm gestations(*Obstet Gynecol* 1996; 87: 231-7 and *Obstet Gynecol* 1996; 88: 1034-40). Yet, there is a paucity of information regarding whether low AF WBC count could be a reassuring sign for an intra-amniotic cavity free of inflammation and respiratory distress syndrome(RDS) of newborns even in patients with preterm labor and intact membranes (PTL) and HCA. The purpose of this study was to examine the frequency and clinical significance of HCA in patients with PTL and low AF WBC counts.

STUDY DESIGN : Amniocentesis was performed in patients with PTL(gestational age < 35.7 weeks). AF was cultured for aerobic and anaerobic bacteria and genital mycoplasmas and assayed for matrix metalloproteinase-8(MMP-8). IAI was defined as an elevated AF MMP-8 concentration(≥ 23 ng/ml). The relationship between HCA and adverse pregnancy outcomes (APO) was examined among consecutive 220 patients with low AF WBC counts(defined as an AF WBC count < 19 cells/mm3). APO included IAI and RDS of newborns. Nonparametric tests were used for statistical analysis.

RESULTS : 1) HCA was present in 24.5%(54/220) in patients with PTL and low AF WBC counts;2) Patients with HCA had significantly higher rates of IAI and RDS of newborns than those without HCA(IAI, 68.0 % vs. 22.4 %; RDS, 30.0 % vs. 8.1 %; p<.005 for each), and this difference remained significant after adjustment for gestational age at amniocentesis;3) Patients with HCA had a significantly higher median AF MMP-8 concentration than those without HCA(41.0 ng/ml [0.3-553.3 ng/ml] vs. 6.4 ng/ml, [0.3-307.3 ng/ml], P<.00005).

CONCLUSION: HCA is present in approximately one-fourth of patients with PTL and low AF WBC counts and is a significant risk factor for IAI and RDS.

(1) Obstet Gynecol 1996; 87: 231-7
(2) Obstet Gynecol 1996; 88: 1034-40

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GESTATION LENGTH AND FETAL GROWTH HAVE DIFFERENT EFFECTS ON CORTICOSPINAL EXCITABILITY AND MOTOR SKILL DEVELOPMENT IN CHILDREN.

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OBJECTIVES

Children born preterm (i.e. < 37 weeks gestational age [GA]) often exhibit motor dysfunction at school age when compared to their term-born peers suggesting preterm birth alters normal corticospinal development. We aimed to differentiate the effects of GA and sub-optimal fetal growth i.e. low birthweight centile (BW%) on corticospinal tract and motor skills development in non-cerebral palsy children.

METHODS

Transcranial magnetic stimulation and surface electromyography were used to evoke corticospinal motor potentials (MEPs) in 132 children (aged 11.9 ± 0.8 years) who were born 28 – 41 weeks GA. BW% (42.1 ± 0.8%) was calculated using the GROW centile calculator. MEP stimulation thresholds (MT) and stimulus-response curves were obtained for left and right motor cortex (M1) projections to an intrinsic hand muscle. Motor function was assessed with the Movement Assessment Battery for Children (MABC-2).

RESULTS

At least one MT was obtained from all children. Shortened GA was linearly associated with increased MT in both hemispheres but low BW% was independently associated with increased MT only in the right M1 ($r = -0.31$, $p = 0.008$, $N = 78$). Stimulus-response curves could be obtained from only 70 children and, apart from MT, no other significant effect of GA or BW% was evident. MABC-2 scores correlated negatively with MT but not GA or BW%.

CONCLUSIONS

Even in the mildly preterm, reduced GA and BW% are associated with reduced corticospinal excitability that is still evident in late childhood. Shortened GA affects both cortices, while low BW% preferentially reduces right M1 excitability, suggesting that brain sparing in fetal growth restriction may be hemisphere-specific. Motor skill development appears influenced more by post-natal cortical development than preterm birth *per se*. Our findings may underestimate the effects of GA and BW% as children with the low cortical excitability could not be fully assessed with TMS.

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DOSE RESPONSE EFFECT OF DOCOSAHEXANOIC ACID SUPPLEMENTATION ON PLASMA MARKERS OF OXIDATIVE STRESS IN PRETERM NEONATES (28-32 WEEKS) FROM INITIATION OF ENTERAL FEEDS TO DAY 28 OF LIFE

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Background: We have demonstrated that docosahexanoic acid (DHA) supplementation improves neonatal cognitive and clinical outcomes even with erythrocyte membrane DHA levels lower than those of term infants. Whilst higher DHA supplementation could further reduce neonatal morbidity, concern remains that DHA may result in dose-dependent increases in oxidative stress. With *in vivo* evidence contradictory and with no data specific to preterm newborns the aim of the study was to determine if increasing doses of DHA disrupted normal plasma redox balance.

Methods: A randomised controlled, dose response trial. Infants <33 weeks gestation (n=32) were randomised to receive one of three oral supplements (DHA at 37 mg/kg/day, 76 mg/kg/day or 121 mg/kg/day) within five days of commencing enteral feeds. Serial plasma samples were taken on days 0, 7, 14, 21, and 28 and plasma malondialdehyde (MDA) and nitrotyrosine determined by ELISA. Data was analysed by repeated measures ANOVA.

Results: There were no significant differences in clinical characteristics between the groups. A significant interaction was observed between DHA supplementation group and time (p=0.005). Post-hoc analysis demonstrated a significant reduction in plasma MDA over the 28 days for the low DHA group (37mg/kg/day) (p=0.001), with no reduction in either the mid or high dose groups. No significant effect for time or DHA supplementation group was observed for nitrotyrosine but levels in those babies supplemented with the low dose DHA were lower at each time point (p=0.014).

Conclusions: DHA supplementation at a dose designed to approximate in utero accumulation has been shown to reduce long term morbidity in high risk preterm infants. It is attractive to hypothesise that greater supplementation would increase the beneficial effects of this simple intervention. However, with preterm infants at particular risk of oxidative and nitrosative damage, the results of the current study would suggest we should exercise caution.

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SIGNAL TRANSDUCTION AND ACTIVATION OF TRANSCRIPTION FACTOR 3 (STAT3) MEDIATES NEONATAL HYPOXIC ISCHAEMIC BRAIN INJURY

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Background: Hypoxia-ischemia (HI) is a major cause of neonatal brain injury. Although a number of biochemical cascades have been implicated, the downstream targets, at the level of transcriptional regulation still remain unclear. The signal transduction and activator of transcription factor 3 (STAT3) is strongly upregulated following peripheral and central trauma and is thus a possible candidate. *Aim:* to investigate the regulation and functional role of STAT3 in neonatal HI brain injury. *Methods:* 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. We used cell-specific conditional knock-out mice (n=6) where STAT3 was deleted in neurons using Synapsin-driven Cre-recombinase in homozygous STAT3-flox mutant mice. Those were compared to littermate controls that were homozygous for STAT3-flox but did not carry the Cre-recombinase (n=5). The assessment of outcome was based on size of infarct (Nissl), extent of cell death (TUNEL density), microglial activation (alphaM&X levels) and astroglial activation (GFAP immunoreactivity). *Results:* at an immunohistochemical level, HI resulted in transient upregulation of phosphorylated STAT3 (Y705) in cortical, hippocampal and thalamic neurons, with a peak at 2-4 hours after 30 min and 8-16 hours after 60 min insult. Moreover, neuron-specific deletion of STAT3 resulted in a significant and strong reduction in infarct size (50-80%, $p<5\%$, t-test), cell death (80-90%, $p<5\%$, t-test), microglial activation (80% reduction of alphaM levels and 70% reduction of alphaX counts, $p<5\%$ t-test) and GFAP immunoreactivity (25%, $p<5\%$, t-test) in hippocampus, cortex, striatum, and thalamus following the severe, 60 min insult. A more moderate effect was also observed in the subcortical white matter. *Conclusion:* Since Y705-phosphorylation plays an important role in STAT3 function, the use of direct kinase inhibitors could serve as a candidate target for therapeutic intervention in neonatal brain damage.

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EXPRESSION OF ANGIOPOIETIN-1 & 2 IN THE MID-GESTATION FETAL SHEEP BRAIN FOLLOWING IN UTERO GLOBAL HYPOXIA.

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Backgound: Antepartum hypoxia can contribute to perinatal brain injury, including vascular leakage and haemorrhage. The inherent vulnerability of periventricular white matter (PVWM) to injury is not well understood, but immaturity of the cerebrovasculature may be important. Angiopoietin [Ang]-1 and 2 are key mediators of blood vessel development. In adult brain, Ang-1 mediates maturation and stabilization of VEGF-induced angiogenesis after ischemia by promoting recruitment of smooth muscle cells to newly formed vessels; Ang-2 (a natural antagonist of Ang-1) leads to rapid angiogenesis and capillary destabilization. Whether similar responses occur in the fetal brain following in utero global hypoxia has not yet been investigated. Aim: This study assessed expression of Ang-1 & Ang-2 in fetal sheep brain following in utero hypoxia caused by umbilical cord occlusion (UCO). Methods: At 95 days gestation (term=147 days), fetal sheep were implanted with an inflatable umbilical cuff. Five days later, severe fetal hypoxia was produced by UCO for 25 mins. Brains were collected 24 hours later from control (cuff not inflated) and UCO fetuses and immersion fixed with 4% paraformaldehyde. Immunohistochemistry was carried out on 12 µm sections using rabbit polyclonal antibodies for Ang-1 and Ang-2 and visualized with metal-enhanced diaminobenzydine. Expression angpt-1 and 2 associated with blood vessels was quantified under light microscopy. Results: UCO increased Ang-1 expression (+22%) and Ang-2 expression (+91%) in PVWM (n=5). Eosin/hematoxylin stained revealed microbleeds in PVWM in 4/5 UCO brains 24 h after hypoxia, while no microbleeds were observed in control brains. Conclusion: These results show that brief, but severe fetal hypoxia increased Ang-1 & 2 expression in the white matter of the pre-term fetal sheep brain. The presence of microhaemorrhages suggests that vascular microstructure becomes unstable after UCO, perhaps as a result of the relatively greater expression of Ang-2.

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COMPENSATORY GROWTH OF HEALTHY CARDIOMYOCYTES IN DISEASED FETAL HEARTS: EFFECTS ON CARDIOMYOCYTE NUMBER AT BIRTH

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Background: It is important to understand how the developing heart responds to insults *in utero* because cardiomyocytes cease proliferating soon after birth when they become terminally differentiated. Hence a reduced complement of cardiomyocytes at birth reduces the life-long functional reserve of cardiomyocytes. A mouse model exhibiting heart-specific inactivation of *Hccs* has been developed. *Hccs* is an X-linked gene encoding Holocytochrome C synthase which is essential in mitochondrial respiration. In heterozygous *Hccs*-knockout females, 50% of their cardiomyocytes are dysfunctional at mid-gestation due to the mitochondrial defect. Although their hearts are fully functional at birth, it is not known whether they are able to fully compensate for 50% of damaged cardiomyocytes in terms of cardiomyocyte number. Hence, this study aimed to examine how the mouse heart responds to damage to 50% of cardiomyocytes during mid-gestation. Method: At birth, cardiomyocyte number was estimated in *Hccs*-knockout and age-matched control hearts using an optical disector-fractionator approach, cardiomyocyte size was determined by measuring cardiomyocyte cross-sectional area whilst cardiomyocyte proliferation was detected by immunofluorescence staining for Ki-67. Results: At birth, body weight was not significantly different between the groups whilst absolute and relative heart weight and volume were significantly reduced in the knockouts. Importantly, the number of cardiomyocytes was significantly reduced in the knockout hearts when compared to controls at birth and this was accompanied by a significant increase in cardiomyocyte size. In addition, cardiomyocyte proliferation was significantly downregulated in knockout hearts when compared to controls at birth. Conclusions: Disease in 50% of cardiomyocytes at mid-gestation is associated with decreased proliferation, a decreased number of cardiomyocytes and compensatory cardiomyocyte hypertrophy at birth. This cardiomyocyte deficit may adversely impact on postnatal cardiac function.

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PROGESTERONE WITHDRAWAL INDUCES INTRAUTERINE PGHS-1 EXPRESSION IN THE PREGNANT GUINEA PIG

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Parturition in the guinea pig occurs in the presence of high circulating progesterone levels and is not delayed by exogenous progestin. We have previously reported that progesterone receptor (PR) levels decrease significantly in guinea pig myometrium during late gestation, coinciding with the induction of amniotic expression of prostaglandin H synthase-1 (PGHS-1). We hypothesised that a positive feed-forward loop exists between functional progesterone withdrawal and PG synthesis in the pregnant uterus. To examine the effect of progesterone withdrawal on intrauterine PG synthesis, pregnant guinea pigs were treated with a PR antagonist (mifepristone; 30 mg/kg/day s.c.) or vehicle (n = 8 each) on days 44, 45 and 46 of pregnancy (term is 65-70d). Amnion, visceral yolk sac (VYS) and placental tissues were collected on day 47 and PGHS-1 protein levels measured by western blotting. PGHS-1 expression was significantly increased in amnion from mifepristone-treated guinea pigs compared to controls (P = 0.0044; t-test), and there was a trend for increased PGHS-1 expression in placenta from mifepristone-treated animals (P = 0.052). PGHS-1 expression was decreased in the VYS of mifepristone-treated animals (P = 0.0042). Levels of the PG inactivating enzyme, 15-hydroxyprostaglandin dehydrogenase (PGDH), were not different in VYS and placenta from mifepristone-treated animals compared to controls, and were undetectable in the amnion. To examine the effect of exogenous PG administration on PR expression, pregnant guinea pigs were treated with the PGE2 analog Sulprostone (0.25 mg) or vehicle (n = 8 each) on day 45, and myometrium was collected 16 hr later. Myometrial PR-A levels were significantly decreased in Sulprostone-treated animals (P = 0.0168) and PR-B also tended to decrease. These data indicate that progesterone withdrawal in the pregnant guinea pig promotes up-regulation of the intrauterine PG production, which promotes further progesterone withdrawal in a feed-forward manner culminating in labour and delivery.

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