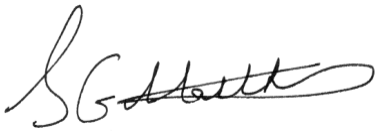
Welcome

Welcome to **Developmental & Perinatal Biology 2018!** This is the **22nd Annual Exchange** in developmental and perinatal biology between The University of Toronto and The Karolinska Institute. The research course has been developed to provide a broad-based interdisciplinary training for graduate students, research fellows, clinical fellows and residents in the area of developmental biology from both basic science and clinical perspectives. The workshop combines a lecture/seminar program with an active research component. The course is also offered as a part of a Graduate course (PSL1080H) at the University of Toronto and as a Graduate course at the Karolinska Institute.

In 2017, the course was held at the Karolinska Institute in Sweden, organized by Ola Hermanson with assistance from Aileen Gracias for the social program. It was attended by 5 Faculty and 16 Trainees from the University of Toronto. From the attendance and success in previous years, it has been clear that there is great interest in this type of summer course. This year we have experienced similar enthusiasm, with 5 Faculty and 12 Trainees attending from Sweden along with 23 Trainees from The University of Toronto. In addition, a large number of Faculty from across the University of Toronto will contribute to the course.

The organization of this type of course requires a considerable input of energy. Therefore, we would like to take this opportunity to thank those on the organizing committee for helping to put the exciting course program together, and to Fredrik Lanner for co-ordinating the Swedish side of the exchange. We would also like to thank Victoria De Luca, Eva Eng and Liz Eng who have provided invaluable organizational support. Finally, we would like to express our gratitude to the Department of Physiology (University of Toronto), our sponsors, many of whom have provided continuous support over the last 22 years, and who have made **Developmental & Perinatal Biology 2018** possible.

Please accept our warmest welcome to what we hope will be an exciting academic and social experience.



S. G. Matthews
Professor
Physiology, Ob/Gyn & Medicine
University of Toronto

Brian Cox, PhD
Associate Professor
Department of Physiology
University of Toronto

Local Organizing Committee:

Stephen Matthews (Chair)
Brian Cox
Victoria De Luca
Liz Eng (trainee)
Evelyn Lambe
Stepehn Lye
Patrick McGowan
Cristina Nostro
Martin Post
Janet Rossant

Co-Sponsors:

Department of Physiology, University of Toronto
Lunenfeld-Tanenbaum Research Institute, Sinai Health System
Department of Obstetrics & Gynaecology, University of Toronto
Hospital for Sick Children, Research Institute
Faculty of Medicine, University of Toronto
Heart and Stroke/Richard Lewar Centre of Excellence in Cardiovascular Research
Mats Sundin Foundation

Location:

Registration:	**August 27** – Room 2172, Medical Sciences Building, University of Toronto
Lectures:	**August 27-31** – Room 2172, Medical Sciences Building, University of Toronto
Practical Workshops:	The Centre for Phenogenomics (TCP) Medical Sciences Building, University of Toronto NICU at Mount Sinai Hospital Centre for Commercialization of Regenerative Medicine (CCRM) Lunenfeld-Tanenbaum Research Institute (LTRI) CReATe Fertility Centre

Social Activities:

August 27	Baseball Game: Toronto Blue Jays vs Baltimore Orioles, The Rogers Centre
August 28	Course Welcome Reception, Faculty Club, University of Toronto
August 29	Toronto Island Picnic
August 30	Canadian National Exhibition
August 31	Friday Night at the Royal Ontario Museum

FACULTY—University of Toronto:

	Department	Location
Hanna Balakier		CReATe Fertility Centre
Isabella Caniggia	Ob/Gyn, Physiology and Medical Sciences	Lunenfeld-Tanenbaum Research Institute (LTRI)
Brian Cox	Physiology	University of Toronto
Paul Delgado Olguin	Molecular Genetics	Hospital for Sick Children
Yenge Diambomba		NICU, Mount Sinai Hospital
Caroline Dunk	Ob/Gyn	Lunenfeld-Tanenbaum Research Institute
Jim Eubanks	Neuroscience	Toronto Western Research Institute
Marina Gertsenstein		The Centre for Phenogenomics
Brian Kavanagh	Anesthesia	Hospital for Sick Children
Michael Laflamme	Laboratory Medicine and Pathobiology	Toronto Western Research Institute
Evelyn Lambe	Physiology	University of Toronto
Jennifer Law		RCWIH BioBank
Julie Lefevbre	Neuroscience	Hospital for Sick Children
Clifford Librach	Ob/Gyn	CReATe Fertility Centre
Stephen Lye	Ob/Gyn and Physiology	Lunenfeld-Tanenbaum Research Institute
Svetlana Madjunkova		CReATe Fertility Centre
Michael May		Centre for Commercialization of Regenerative Medicine (CCRM)
Stephen Matthews	Physiology and Ob/Gyn	University of Toronto and LTRI
Patrick McGowan	Biological Sciences	University of Toronto at Scarborough
Lubna Nadeem	Ob/Gyn	Lunenfeld-Tanenbaum Research Institute
Angela Neish		NICU, Mount Sinai Hospital
Cristina Nostro	Physiology	University of Toronto
Monica Pereira		The Centre for Phenogenomics
Eszter Posfai	Molecular Biology	Hospital for Sick Children
Martin Post	Paediatrics & Physiology	Hospital for Sick Children
Amy Ramsey	Pharmacology & Toxicology	University of Toronto
Janet Rossant	Molecular Genetics & Ob/Gyn	Hospital for Sick Children
Stewart Russell		CReATe Fertility Centre
Julien Sallais	Ob/Gyn	Lunenfeld-Tanenbaum Research Institute
John Sled	Biomaterials and Biomedical Engineering, Ob/Gyn	Hospital for Sick Children
Parshvi Vyas		CReATe Fertility Centre
Tom Waddell	Thoracic Surgery	Princess Margaret Cancer Centre
Frances Wong	Physiology	University of Toronto

Karolinska Hospital/Institute:

Ola Hermanson	Neuroscience
Fredrik Lanner	Clinical Science, Intervention and Technology
Ron Li	Reparative Medicine
Kristiina Tammimies	Neuropsychiatry
Anna Wredenberg	Molecular metabolism

Monday, August 27

8:30 Registration with breakfast — Room 2172, Medical Sciences Building, University of Toronto

8:45 Welcome/Introduction — **Stephen Matthews**, International Exchange Program for Developmental and Perinatal Biology, University of Toronto

Stem Cells, Embryonic Development and Disease

Co-ordinators: Janet Rossant and Cristina Nostro

9:00 **Eszter Posfai:** Building an early mouse embryo *in vivo* and *in vitro*

9:40 **Fredrik Lanner:** Early human development and embryonic stem cells

Coffee

10:40 **Cristina Nostro:** Developing hESC-derived pancreatic cells for diabetes treatment

11:20 **Michael LaFlamme:** Heart regeneration with pluripotent stem cells

Trainee Presentations (Orals 1-3)

12:00 **Chenhong Lin[1]:** Human organotypic spinal cord slice culture as a useful model to study human injury mechanisms, therapeutic strategies & biomaterials

12:15 **Nerges Winblad[2]:** HLA knockout in human embryonic stem cells

12:30 **Xiaoyuan Ren[3]**: Redox regulation in vertebrate embryonic development

13:00 **Lunch** - MSB Cafeteria

Research Workshops:
14:00-17:00 1) Generation of genetically modified mouse models
13:30-15:30 2) Introduction to R and graphics for biology

18:00 **Baseball Game - Toronto Blue Jays vs Baltimore Orioles, The Rogers Centre**

Tuesday, August 28

Placenta and Birth

Co-ordinator: Brian Cox

9:00 **Brian Cox:** Investigating placenta development and pathology with gene expression data

9:40 **John Sled:** Placenta insufficiency and brain injury: cerebral oxygen regulation in the fetus

Coffee

10:40 **Lubna Nadeem:** The molecular triggers of human labor

11:20 **Isabella Caniggia:** Die another way

Trainee Presentations (Orals 4-6)

12:00 **Melanie Audette[4]:** Parental ethnicity and placental maternal vascular malperfusion pathology in healthy nulliparous women

12:15 **Leslie Proctor[5]:** The association between hypertensive disorders of pregnancy and birthweight in twins: the importance of using twin-based growth curves

12:30 **Yelin Zeng[6]**: Wnt16 regulates chondrocyte differentiation through Wnt/ planar cell polarity (PCP) pathway

13:00 **Lunch** - MSB Cafeteria

Research Workshops:	14:00-17:00	1) Generation of genetically modified mouse models
	14:00-15:30	2) NICU Visit at Mount Sinai Hospital
	13:30-15:00	3) Centre for Commercialization of Regenerative Medicine (CCRM)

19:00 **Course Welcome Reception - Faculty Club, University of Toronto**

Wednesday, August 29

Lung and Heart Development

Co-ordinator: Martin Post

9:00 **Tom Waddell:** Regenerative medicine approaches to lung and airway disease

9:40 **Brian Kavanagh:** Negative abdominal pressure ventilation

Coffee

10:40 **Ron Li:** Generating cardiac tissues from pluripotent stem cells

11:20 **Paul Delgado Olguin:** Epigenetic Programming of adult-onset heart disease predisposition by obesity during pregnancy

Trainee Presentations (Orals 7-9)

12:00 **Patrick Chan[7]:** Functional reprogramming of human embryonic stem cell-derived ventricular cardiomyocytes into pacemaker cells

12:15 **Robert D'Cruz[8]:** PTCH1-deficiency in murine FOXD1+ stromal cells causes renal hypoplasia via a non-cell-autonomous mechanism

12:30 **Brahmdeep Saini[9]**: Fetal hemodynamic response to acute maternal hyperoxygenation in the setting of intrauterine growth restriction

13:00 **GROUP PHOTO: please assemble outside on steps of MSB facing King's College Circle**

13:15 **Lunch and Poster Session:** Stone Lobby, MSB 2171

15:00 **Toronto Island Picnic**

Thursday, August 30

Neurodevelopment

Co-ordinator: Evelyn Lambe

9:00 **Kristiina Tammimies:** Genetic decoding of neurodevelopmental disorders and brain development

9:40 **Julie Lefevbre:** TBC

Coffee

10:40 **Jim Eubanks:** Anti-oxidant treatment improves phenotypic deficits in a mouse model of rett syndrome

11:20 **Amy Ramsey:** Adult rescue of NMDA receptors in a mouse model of GRIN1 encephalopathy

Trainee Presentations (Orals 10-12)

12:00 **Elizabeth Eng**[10]: Fetal glucocorticoid exposure modifies the blood-brain barrier after birth

12:15 **Dagmara Kaczynska**[11]: Precise calculations of Iba-1 positive cells in cerebral hemispheres of mice

12:30 **Rola Hammoud**[12]: The role of choline in gestational diets on hypothalamic food intake regulation in wistar rat offspring

13:00 **Lunch** - MSB Cafeteria

Research Workshops:
13:30-15:00 1) Tissue Biobanking in Reproductive Biology
13:30-15:00 2) Methods and Tools to Assess Embryo Quality in the IVF clinic

18:00 **Canadian National Exhibition**

Friday, August 31

Developmental Origins of Health and Disease

Co-ordinators: Stephen Matthews and Patrick McGowan

9:00 **Anna Wredenberg:** Early metabolic disorders linked to the mitochondria

9:40 **Ola Hermanson:** Transcriptional mechanisms involved in brain evolution, development and disease

Coffee

10:40 **Stephen Matthews:** Fetal exposures and long-term health outcomes

11:20 **Patrick McGowan:** Developmental programming of the epigenome

Trainee Presentations (Orals 13-14)

12:00 **Aya Sasaki**[13]: Synthetic glucocorticoids during pregnancy and epigenetic outcomes in the brain and blood of offspring in a guinea pig model

12:15 **Emanuela Pannia**[14]: The effects of folate dose and form during pregnancy on central and peripheral energy balance systems in Wistar rat dams and offspring

12:30 **Lunch** - MSB Cafeteria

18:00 Friday Night at the Royal Ontario Museum

Research Workshop Assignments:

Title: Generation of genetically modified mouse models

Leaders: **Marina Gertsenstein**, **Monica Pereira**, TCP Model Production Core (Transgenic)

Location: **The Centre for Phenogenomics (TCP)**, 25 Orde St. Front lobby security.

Date/Time: This is a two-day hands on work shop, you must attend both days.
Monday-Tuesday August 27 and August 28, 2018; 2:00-5:00 pm each day

NOTE: ***TCP is an exclusion barrier mouse facility that operates with a strict pathogen exclusion policy. For these reasons, entry into the Animal Facility will not be permitted if you have been in any other animal facility, or had exposure to rodents, in the past 48 hours. If these criteria cannot be met, a wet shower must be taken in the ground floor change room before entry.***

Abstract: Introduction to technologies used for the production of genetically modified mouse models. Participants will have the opportunity to collect, manipulate and culture pre-implantation stage mouse embryos under the stereomicroscope and practice aggregation technique used for generation of chimeras with Embryonic Stem (ES) cells.

Day 1
- Presentation describing the current methods
- Demo: morula collection, zona removal, aggregation of embryos with ES cells
- Hands on: aggregation of embryos with ES cells for generation of chimeras

Day 2
- Examination of aggregates after overnight culture
- Demo and Hands on: Collection of blastocysts
- Demo: GFP and RFP mice
- Q & A

STUDENTS:

- Rola Hammoud (UT)
- Anand Lakhani (UT)
- Andrada Naghi (UT)
- Emanuela Pannia (UT)
- David van Bruggen (KI)
- Patrick Chan (KI)
- Chenhong Lin (KI)
- Xiaoyuan Ren (KI)
- Angelo Salazar (KI)

Title: Introduction to R and graphics for biology

Leaders: **Frances Wong**, PhD Candidate and **Brian Cox**, PhD, Department of Physiology, University of Toronto

Location: **Medical Sciences Building,** 1 King's College Circle, Rm 3287 (computer lab is kept locked, please knock when you get there and someone will let you in)

Date/Time: Monday August 27, 2018; 1:30- 3:30 pm

Abstract: R is an open source programming language that was designed for statistical computing but is also equiped with a suite of packages for Biology applications. R strives to promote reproducibility in data analysis and representation making this an invaluable tool, especially with the increasing volumes of research data from RNA-sequencing and genomics.

This workshop will be an introduction to the fundamental principles of R from basic syntax to generating customized graphics (data analysis is a separate topic that will not be covered in this workshop). No R or programming experience required. The workshop aims to get participants comfortable loading in data tables and making figures such as barplots and heatmaps. Participants are invited to bring their own data table to try that would otherwise be graphed in Excel or Prism Graphpad.

STUDENTS:

- Melanie Audette (UT)
- Elizabeth Eng
- Bona Kim (UT)
- Guinever Imperio (UT)
- Connor Mabbott (UT)
- Christina Ricci (UT)
- Brahmdeep Saini (UT)
- Aya Sasaki (UT)
- Katryna Stronks (UT)
- Aijiao Xiao (UT)
- Jiawei Xu (UT)
- Roberto Ballarino (KI)
- Dagmara Kaczynska (KI)
- Viktoria Knoflach (KI)
- Yu Pei (KI
- Nerges Winblad (KI)
- Zeng Yelin (KI)

Title: NICU Visit at Mount Sinai Hospital

Leaders: **Yenge Diambomba**, Staff Neonatologist and **Angela Neish**, NICU Quality-Safety Nurse

Location: **Mount Sinai Hospital**, 600 University Avenue, 18th floor, Room 18-257

Date/Time: Tuesday August 28, 2018; 2:00-3:30 pm

Mount Sinai Hospital is the home of one of the busiest perinatal services in Canada with more than 7000 deliveries, many of which are high risk. The NICU admits about 1100 newborns per year with more than 300 being less than 1500g. Participants will receive a tour of the Neonatal Intensive Care Unit (NICU), followed by a discussion on relevant neonatal clinical care topics such as family-integrated care, care of the peri-viable infant and patient safety practices in the NICU at Mount Sinai Hospital.

STUDENTS:

- Elizabeth Eng
- Bona Kim (UT)
- Brahmdeep Saini (UT)
- Katryna Stronks (UT)
- Frances Wong (UT)
- Aijiao Xiao (UT)
- Jiawei Xu (UT)
- Yu Pei (KI)
- Zeng Yelin (KI)

Title: Centre for Commercialization of Regenerative Medicine (CCRM)

Leaders: **Michael May** PhD, President and CEO

Location: **MaRS West Tower**, Suite 1002, 661 University Ave. Toronto, M5G 1M1

Date/Time: Tuesday August 28, 2018 from 1:30 pm to 3 pm

Presentation Title: CCRM: Collaborative, Capital-Efficient Commercialization of Regenerative Medicine

CCRM, a Canadian not-for-profit organization funded by the Government of Canada, the Province of Ontario, and leading academic and industry partners, supports the development of regenerative medicines and associated enabling technologies, with a specific focus on cell and gene therapy. A network of academic researchers, leading companies, strategic investors and entrepreneurs, CCRM aims to accelerate the translation of scientific discovery into new companies and marketable products for patients, with specialized teams, funding and infrastructure. CCRM sources and evaluates intellectual property from around the globe, offers various consulting services, conducts development projects with partners, and establishes new companies built around strategic bundles of intellectual property. CCRM has a fully resourced development facility used to both evaluate and advance technologies, a Centre for Advanced Therapeutic Cell Technologies and a (coming soon!) GMP facility within a 40,000 square foot office designed for advanced cell manufacturing. CCRM is the commercialization partner of the Ontario Institute for Regenerative Medicine and the University of Toronto's Medicine by Design. CCRM is hosted by the University of Toronto and was launched in Toronto's Discovery District on June 14, 2011. Visit us at www.ccrm.ca

STUDENTS:

- Melanie Audette (UT)
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- Connor Mabbott (UT)
- Christina Ricci (UT)
- Roberto Ballarino (KI)
- Dagmara Kaczynska (KI)
- Aya Sasaki (UT)
- Nerges Winblad (KI)

Title: Tissue Biobanking in Reproductive Biology

Leaders: **Caroline Dunk**, PhD, Research Associate, LTRI
Julien Sallais, PhD Candidate, LTRI
Jennifer Law, MSc, RCWIH BioBank Manager

Location: **Lunenfeld-Tanenbaum Research Institute (LTRI),** 60 Murray Street, Room 6-1013

Date/Time: Thursday August 30, 2018; 1:30-3:00 pm Director of Reproductive Genetics,

Abstract: Biobanks are utilized by researchers worldwide as a convenient and accessible means of acquiring rare or difficult to obtain biological samples for use in research. At the Research Centre for Women's and Infants' Health (RCWIH) BioBank, our focus is on collecting and distributing ethically-approved, pregnancy-related biological specimens and comprehensive clinical data. Perinatal specimens have applications in research beyond pregnancy and may be utilized in diverse fields such as developmental biology, cancer cell biology, regenerative medicine, and stem cell research. The biological samples and associated clinical data that are provided through biobanks serve as a foundation for translational research programs and establish a strong link between laboratory-derived results and the clinical conditions under investigation. During this workshop, we will discuss the utility of biobanks in today's research and how they are operated and accessed. We will also provide the opportunity to view a human placenta in early and late gestation, as well as the umbilical cord and membranes, and will use them to demonstrate tissue collection techniques. This workshop will provide insight into the daily routine used to collect samples for the RCWIH BioBank's archive, and how these samples facilitate translational research.

STUDENTS:

- Elizabeth Eng (UT)
- Connor Mabbott (UT)
- Rola Hammoud (UT)
- Guinever Imperio (UT)
- Christina Ricci (UT)
- Aya Sasaki (UT)
- Katryna Stronks (UT)
- Jiawei Xu (UT)
- Patrick Chan (KI)
- Dagmara Kaczynska (KI)
- Viktoria Knoflach (KI)
- Chenhong Lin (KI)
- Yu Pei (KI)
- Xiaoyuan Ren (KI)
- Nerges Winblad (KI)
- Zeng Yelin (KI)

Title: Methods and Tools to Assess Embryo Quality in the IVF clinic

Leaders: **Clifford Librach** (MD), Medical and Scientific Director, CReATe Fertility Centre
Svetlana Madjunkova (MD, PhD), Dir. of Reproductive Gen., CReATe Fertility Centre
Stewart Russell, Director of Implantation Biology Research, CReATe Fertility Centre
Hanna Balakier, Director of Embryology, Create Fertility Centre
Parshvi Vyas (MSc), Projects Manager, CReATe Fertility Centre

Location: **CReATe Fertility Centre**, 790 Bay Street, Suite 1100

Date/Time: Thursday August 30 2018, 1:30-3:00 pm

Abstract: Within this workshop, attendees will be introduced to assisted reproductive technologies, focusing on *in vitro* fertilization (IVF) and cutting-edge methods that are currently available and applied at CReATe for the selection of IVF embryos with the greatest chance of successful implantation. Recent advances and ongoing research in pre-implantation genetic testing and non-invasive embryo competency assessments will be presented. Participants will have the opportunity to tour the fertility clinic and laboratories and to interact one-on-one with experts in the field of human embryology and reproductive genetics.

STUDENTS:

- Melanie Audette (UT)
- Christopher Casciaro (UT)
- Bona Kim (UT)
- Anand Lakhani (UT)
- Andrada Naghi (UT)
- Emanuela Pannia (UT)
- Brahmdeep Saini (UT)
- Aijiao Xiao (UT)
- Roberto Ballarino (KI)
- David van Bruggen (KI)
- Angelo Salazar (KI)

ABSTRACTS

Orals

O1

Human organotypic spinal cord slice culture as a useful model to study human injury mechanisms, therapeutic strategies & biomaterials

Lin C[1], Calzarossa C[1,2], Liu J [1,3], Fernandez-Zafra T[4], Holmberg L[1], Rising A[1,5], Johansson J[1], Andersson M[5], Vazquez-Juarez E[1],Lindskog M[1], Uhlén P[4] and Åkesson E[1,6]

[1] Department of Neurobiology, Care Sciences and Society, Karolinska Institutet, Stockholm, Sweden,
[2]NeuroZone s.r.l. Milan, Italy,
[3]Department of Neurology, First Hospital of Jilin University, Changchun, P.R. China,
[4]Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden,
[5]Department of Anatomy, Physiology and Biochemistry, Swedish University of Agricultural Sciences, Uppsala, Sweden
[6]R&D unit, Stockholms Sjukhem, Stockholm, Sweden.

Human nervous system lesions, such as spinal cord injury (SCI), often affect young persons, leading to extensive functional deficits. No clinical treatment offering functional improvement exists. Numerous promising preclinical studies including compounds, cell therapy and/or training have led to clinical trials. However, translation from animal models to the clinic has proven challenging. Species differences may be one explanation. To develop clinically potent and safe treatments and translate proof of concept findings in animal models to clinical settings, we also need models to study human species-specific injury and repair mechanisms.

We established an *ex vivo* human spinal cord slice culture model with human first trimester tissue after informed consent by tissue donors and ethical approval from EPN, Medicine, Stockholm. We have validated the tissue viability and organotypic features through morphological study, immunohistochemistry, calcium imaging and electrophysiological recording after being cultured for 21 days *in vitro* (DIV). We also proved that this *ex vivo* model is a good platform to study human spinal cord injury and evaluate the therapeutic potential of allogenic human neural progenitor cell (hNPC) transplantation as well as novel biomaterials such as artificial spider silk. At 14 and 21 DIV, we observed an increased number of caspase-3^+ cells and activated microglia in response to contusion injury in the slices compared to controls. However, a significantly reduced apoptosis and microglial activation was observed after neural cell therapy to injured slices.

Artificial biomimetic spider silk fiber was studied in these human spinal cord derived cultures in vitro by analyzing cell proliferation, migration, differentiation and neurite outgrowth. Our preliminary data suggest that human spinal cord-derived cells migrate and extend neurites along artificial biomimetic spider silk fibers.

We conclude, that human spinal cord slice culture is a promising model to study human SCI and therapeutic strategies including novel biomaterials.

O2

HLA Knockout in Human Embryonic Stem Cells

Nerges Winblad[1*], Sandra Petrus Reurer[2*] and Fredrik Lanner[1]
1. Department for Clinical Science, Intervention and Technology, Karolinska Institutet
2. Department of Clinical Neuroscience, Karolinska Institutet
* These authors contributed equally to this work

Allogenic transplantations require donor-host HLA-matching as well as immunosuppressive drugs to reduce the risk of rejection. This is mainly caused by mismatched HLA molecules that are recognized as foreign by the host's T lymphocytes, leading to T cell activation and clearing of the graft. The HLA genes are highly polymorphic transmembrane proteins. HLA class I proteins require β-microglobulin for presentation on the cell surface, making it an ideal target for gene editing. Furthermore, upon inflammation in the presence of cytokine interferon gamma (IFN-γ), cells may also upregulate HLA class II molecules, which are recognized by the host immune system causing rejection of the transplanted tissue. We hypothesized that knocking out a key regulator of HLA class II genes would generate a cell type unable to express HLA class II molecules even in the presence of IFN-γ. We exploited the CRISPR/Cas9 technology to edit the well-conserved β-microglobulin gene and the main regulator Class II Major Histocompatibility Complex Transactivator (CIITA) thereby generating human embryonic stem cell (hESC) lines lacking HLA class I and class II. Starting with HLA class I knockout cells, to test whether they were incapable of presenting HLA molecules on the cell surface we sought to characterize different single cell derived-hESC clones to determine if the induced mutation had led to a protein knockout through western blot, immunohistochemistry, qPCR and Fluorescence-Activated Cell Sorting. The resulting genome editing demonstrated that HLA class I surface presentation had been abrogated by the introduced mutation. We used these cells to generate an HLA double-knockout line by targeting CIITA. We are currently functionally characterizing as well as differentiating these lines to validate the HLA double-knockout and to explore their potential for regenerative medicine.

O3

Redox Regulation in Vertebrate Embryonic Development

Xiaoyuan Ren[1], Arne Holmgren[1]
[1]Division of Biochemistry, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden

During the early postimplantation period, rodent embryos develop under a relatively hypoxic condition and they are sensitive to oxygen concentration. When the uteroplacental circulation is established, oxygen level increases dramatically, coinciding with an elevated level of reactive oxygen species (ROS). Studies have revealed that ROS can serve as messenger molecules mediating redox signaling which plays critical roles in various biological events. Accurately exposing embryos to a high level of oxygen benefits the rapid growth and differentiation because ROS mediated apoptosis is essential for normal embryonic morphogenesis. On the other hand, excessive ROS are detrimental due to their reactivity towards biological molecules, such as DNA, lipids and proteins and contribution to developmental pathologies, such as teratogenesis and preeclampsia. To counteract the damage caused by ROS, cells are equipped with antioxidant systems, such as glutathione (GSH) system, thioredoxin (Trx) system, etc. GSH is the most abundant antioxidant thiols in cells and knocking out glutathione synthetase resulted in embryonic death between E7.5-8.5. The Trx system, consisting of thioredoxin reductase (TrxR), Trx and NADPH, is heavily involved in redox regulation of embryonic development. TrxR utilizes NADPH to reduce Trx that sequentially reduces its downstream substrates. Knocking out cytosolic Trx (Trx1) is embryonically lethal in the early postimplantation period around E7.5, showing a significantly reduced proliferation of inner mass cells. While, the embryos from cytosolic TrxR (TrxR1) knockout mice died around E10.5, exhibiting growth retardation and abnormal tissue

development. Interestingly, there is a time gap between Trx1 and TrxR1 knockout embryos, suggesting antioxidant defense is not the only mechanism of the Trx system mediated regulation in embryonic development. Therefore, understanding the redox signaling regulated by Trx system may help solve the puzzle.

O4

Parental Ethnicity and Placental Maternal Vascular Malperfusion Pathology in Healthy Nulliparous Women

Melanie C. Audette, MD, PhD [1,2] Ms. Khrystyna Levytska, MSc [3] Stephen J. Lye, PhD [1-3] Nir Melamed, MD [4] John C. Kingdom, MD [1-3,5]

[1.] The Lunenfeld-Tanenbaum Research Institute – Toronto, ON, Canada
[2] Faculty of Medicine - University of Toronto, Toronto, Ontario, Canada
[3] Department of Obstetrics and Gynaecology (Maternal Fetal Medicine), University of Toronto, Toronto, Ontario, Canada;
[4] Maternal-Fetal Medicine Division, Department of Obstetrics and Gynaecology, Sunnybrook Health Sciences Centre;
[5] Department of Laboratory Medicine and Pathobiology, University of Toronto

Disclosure: The authors report no conflict of interest.

Introduction: Rates of some placental-associated pregnancy complications vary by ethnicity, though the strength of association with underlying placental pathology is presently unknown. Our objective was to determine whether an association between ethnicity and placental pathology occurs in low-risk pregnancies. Methods: 829 low-risk nulliparous pregnant women were prospectively studied. Data were obtained from standardized obstetrical appointments (clinical history, serum biomarkers, placental ultrasound) and hospital delivery records (pregnancy complications, delivery details and perinatal outcomes). Placental pathology was performed in all subjects using standard criteria. Results: In our cohort, 72% of women were Caucasian, 14% East Asian, 8% South Asian, 4% Afro-Caribbean and 3% Hispanic women. 81% of couples were concordant (same ethnic background) and 19% discordant (mixed ethnicities). South Asian women had the highest rate of small for gestational age (SGA) birth (customized birthweight <10th percentile) (24.2%), which was associated with the placental features of uteroplacental vascular insufficiency (placental weight <10th percentile with decidual vasculopathy, focal infarction, and/or syncytial knot formation) ($p=0.05$). Placental efficiency varied significantly by ethnicity; Caucasian women had the highest efficiency (7.1±1.2) and Afro-Caribbean women the lowest (6.5±0.9) ($p<0.003$). Afro-Caribbean women had the highest rate of marginal cord insertion. Placental efficiency, was higher in concordant vs. discordant couples (7.0±1.2 vs. 6.8±1.1; $p<0.05$). Placental histopathology was not affected by parental ethnic discordance. Discussion: Maternal ethnicity influences placental efficiency and relationship between uteroplacental vascular insufficiency and SGA birth, but was not associated with other placental pathologies. Discordant parental ethnicity did not affect the development of placental pathologies or adverse pregnancy outcomes.

O5

The Association Between Hypertensive Disorders Of Pregnancy And Birthweight In Twins: The Importance Of Using Twin-Based Growth Curves

Leslie K. Proctor[1], Julia Kfouri[2], Jon F.R. Barrett[3], and Nir Melamed[1,3]
[1]Department of Obstetrics and Gynecology, University of Toronto.
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Hypertensive disorders of pregnancy (HDP) are associated with fetal growth restriction in singleton pregnancies, with abnormal placentation being a likely shared etiology. There is conflicting data regarding whether a similar association is present in twins. We aimed to describe the association between HDP and small-for-gestational-age (SGA) in twins compared to singletons using appropriate birthweight reference. This was a retrospective study of women with twin or singleton pregnancies at a single tertiary centre (2003-2015). SGA was defined as birthweight<10th percentile for gestational age and sex based on either singleton- or twin-based reference. The association of HDP with SGA was compared between twins and singletons, and was adjusted for potential confounding variables. 49,139 singleton and 2,232 twin pregnancies were included. The rate of HDP was higher for twins than singletons (10.7% vs 4.1%, p<0.001). The association of HDP with SGA was stronger among singletons than among twins when SGA was defined using a singleton-based reference (Singletons: 8.9% vs. 3.7%, p<0.001, aOR=2.27 [95%-CI 2.00-2.58]; Twins: 12.6% vs. 9.7%, p=0.038, aOR=1.37 [95%-CI 1.03-1.83]). When a twins-based reference was used to define SGA in the twins group, the association of HDP with SGA among twins (19.3% vs. 9.5%, p<0.001, aOR 2.33 [95% CI 1.64-3.33]) was similar to that observed in singletons. The association of HDP with SGA in twin pregnancies is similar in magnitude to that observed in singletons.

O6

Wnt16 regulates chondrocyte differentiation through Wnt/ planar cell polarity (PCP) pathway

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Wnt signaling, a highly conserved signaling pathway, plays important roles in endochondral ossification which is a key process for skeletal development and bone repair. Wnt16, as one of the nineteen Wnt ligands, is reported to repress osteoclastogenesis, prevent cortical bone fragility fractures and to be upregulated in osteoarthritis. But how Wnt16 mediates chondrocyte differentiation during endochondral ossification is still unclear. Here, we investigate the roles of Wnt16 specifically in chondrocytes during endochondral ossification. First, we generated Col2a1-Wnt16 transgenic mice in which Wnt16 was overexpressed in chondrocytes under the control of Col2a1 promoter and enhancer. The transgenic mice showed a great reduction of tissue mineralization during embryonic development. We also genetically knocked out Wnt16 by generating Wnt16Loxp/Loxp;Col2a1-Cre mutant mice to examine whether Wnt16 is required for skeletal development. The mutant mice showed no severe phenotype in early skeletal development. However, after 2-month-old, the mutant mice displayed a smaller body size and lower bone mass as compared to that of control littermates. In vitro, our studies showed that Wnt16 delays chondrocyte hypertrophy and subsequent maturation. Mechanistically, we found that Wnt16 mainly activates the planar cell polarity (PCP) pathway through activation of JNK in primary chondrocyte. After treated chondroprogenitor cell line ATDC5 with SP600125, a JNK specific inhibitor, Wnt16-induced delay of chondrocyte hypertrophy is eliminated. In addition, our data suggest that Wnt16 mainly interacts with Ror2 or CD146, co-receptors of PCP pathway, but not Vangl2 or Ryk. Collectively, our current study provides evidence that Wnt16 delays chondrocyte hypertrophy through PCP pathway partially by binding to Ror2 and CD146. Our findings deepen the understanding of chondrocyte differentiation during endochondral ossification.

O7

Functional reprogramming of human embryonic stem cell-derived ventricular cardiomyocytes into pacemaker cells

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Normal heart rhythm originates in the sino-atrial node, a specialized cardiac tissue consisting of only a few thousand nodal pacemaker (Pm) cardiomyocytes (CMs). Malfunction of PmCMs due to diseases or aging leads to rhythm generation disorders, necessitating the implantation of electronic Pm, with such shortcomings as limited battery life, permanent implantation of leads, lack of autonomic responses etc. As such, the engineering of bio-artificial Pm (BPm) as an alternative to electronic devices has been pursued. Human pluripotent stem cell (hPSC) can self-renew and differentiate into all lineages, serving as an unlimited CM source. However, the yield of PmCMs is typically poor, independent of hPSC lines; recently, we reported a highly efficient hPSC specification protocol, enabling mass generation of ventricular (V) CMs with ~100% yield. Building upon our series of previous studies of the crucial PmCM protein hyperpolarization-activated cyclic nucleotide-gated channel 1 (HCN1), here we functionally reprogrammed hPSC-VCMs into -PmCMs via adeno-associated virus-mediated overexpression of the engineered HCN1 channel (HCN1ΔΔΔ) whose S3-S4 linker residues 246-248 have been strategically deleted to promote cardiac pacemaking. rAAV9-HCN1ΔΔΔ-reprogrammed hPSC-PmCMs converted from -VCMs showed automaticity and action potential parameters typical of native nodal PmCMs.

O8

PTCH1-deficiency in murine FOXD1+ stromal cells causes renal hypoplasia via a non-cell-autonomous mechanism

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Background: Congenital Anomalies of the Kidney and Urinary Tract (CAKUT) represent a broad range of disorders that cause 30-50% of pediatric cases of chronic kidney disease. Among the molecular pathways that underlie the pathogenesis of CAKUT, our lab has recently demonstrated that aberrant Hedgehog (HH)-GLI signaling in the Foxd1+ stromal lineage disrupts renal development. Yet, the cell-autonomous and non-cell autonomous mechanisms that control HH-GLI-dependent aberrant signaling are not elucidated. The objective of this work is to test our hypothesis that predominantly non-cell-autonomous mechanisms underlie the pathogenesis of HH-GLI-dependent aberrant signaling in the Foxd1+ stromal lineage. Methods: FoxD1Cre;Ptch1+/- mice were crossed with Ptch1loxP/loxP mice to generate mutants with constitutively active stromal HH signaling. Mutant mice (FoxD1Cre;Ptch1fl/-) and littermate controls (Ptch1loxP/+) were analyzed through fluorescence-activated cell sorting and qRT-PCR, histology, immunofluorescent imaging, and murine embryonic kidney culture. Results: Histologic analysis of Ptch1-deficient mice showed that kidneys are phenotypically normal at E13.5 (Fig 1A-B); however renal hypoplasia is observed by E15.5 (Fig 1D-E), and progressively worsens by E18.5 (Fig 1G-H). At E18.5, the cortical stroma is spatially disorganized

(Fig 1G-H), and kidney volume (Fig 1F) and nephron numbers (Fig 1I) are decreased by 48% (n=4, p=0.12) and 41% (n=4, p<0.05) respectively. Immunofluorescent analysis of PBX1 and SIX2 expression in stromal and nephrogenic cells, respectively, revealed that the number of both PBX1-positive and SIX2-positive cells per ureteric bud tip was decreased by 18% (n=4, p<0.05) and 25% (n=4, p<0.05) at E18.5 in Ptch1-deficient mutants. Analysis of stromal cell proliferation using mitosis-specific marker phospho-histone H3 (PHH3) showed no changes in Ptch1-deficient mutants at E13.5 or E15.5; however, a decrease of 41% (n=3, p<0.05) was detected at E18.5. Cell-non-autonomous signaling through the ureteric bud was analyzed through culturing kidney explants at E12.5, and revealed that the number of ureteric bud tips was decreased by 28% in Ptch1-deficient mutants. Conclusions: Together, these results indicate that the primary mechanism underlying renal hypoplasia in stromal-specific Ptch1-deficient mutants is cell-non-autonomous signaling through the ureteric bud. Our results provide a basis to identify molecular mechanisms by which HH-GLI signaling in stromal cells controls ureteric bud branching.

O9

Fetal hemodynamic response to acute maternal hyperoxygenation in the setting of intrauterine growth restriction

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Introduction Placental insufficiency results in chronic fetal hypoxemia that leads to intrauterine growth restriction (IUGR) because oxygen delivery to the fetus is an independent determinant of fetal growth (1). Furthermore, IUGR offspring are at an increased risk of adverse perinatal and long-term outcomes, including neurodevelopmental and cardiovascular complications (2). One approach to preventing these negative effects may be to increase fetal oxygenation via maternal hyperoxygenation (MH), which improves outcomes in severely IUGR pregnancies (3). We present the hemodynamic response to acute MH in normoxemic and chronically hypoxemic IUGR fetuses.

Methods At 109-111 days (d) gestation (term=150d), pregnant Merino ewes (n=6, control; n=4, IUGR) underwent surgery, with general anesthesia induced by intravenous injection of diazepam (0.3 mg/kg) and ketamine (7 mg/kg) and maintained with inhalation of isoflurane (1-2%) in oxygen with post-operative antibiotics and analgesia, to implant vascular catheters in the maternal jugular vein, fetal femoral artery and vein as well as the amniotic cavity. At 126±1d, ewes underwent cardiovascular MRI assessment using a 3T Siemens Skyra system in both a normoxic and hyperoxic (100% O2) state under general anesthesia induced and maintained as above (isoflurane, 2.5-2.75%). MRI assessment was performed using phase contrast (PC) measurements of blood flow in major fetal and placental vessels including uterine arteries, umbilical vein (UV), ductus venosus, ascending aorta (AAO), main pulmonary artery, branch pulmonary arteries (PAs), ductus arteriosus, common carotid arteries (CCAs), superior vena cava and descending aorta. Foramen ovale (FO) shunting was calculated by subtracting flow in PAs from AAO. All blood flow measures were indexed to fetal weight calculated from the 3D MRI acquisition of fetal volume (4).

Results MH significantly increased fetal arterial PaO2 (Control: 19.1+/-1.6 vs 22.7+/-1.4 mmHg; IUGR: 16.1+/-1.7 vs 21.4+/-2.6). IUGR fetuses had significantly reduced uterine artery blood flow in comparison to controls which did not change in response to MH. Acute MH significantly increased blood flow in the fetal PAs and decreased blood flow through the FO whilst other major vessels such as the UV, AAO and CCAs were unaltered. The data was analyzed using a two-way ANOVA (statistical significance, $P<0.05$).

Conclusions We used PC-MRI to perform a comprehensive assessment of the fetal circulation and show that acute MH increases PA and decreases FO blood flow in both controls and IUGR fetuses. Unaltered flow in the UV and CCAs with increased arterial fetal oxygen content suggests increased oxygen delivery to the fetus and the brain. Despite reduced uterine artery blood flow, a chronic MH treatment can be of great benefit to an IUGR fetus to improve oxygen delivery, particularly to important organs like the brain, heart, lungs and liver that may help the timely development and maturation of its vital organs and improve its growth trajectory.

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O10

Fetal Glucocorticoid Exposure Modifies The Blood-Brain Barrier After Birth

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INTRODUCTION: The blood-brain barrier (BBB) is formed by specialized endothelial cells connected by tight-junctions (TJ) to form a non-fenestrated vessel. The TJs together with luminal membrane transporters P-glycoprotein (P-gp; encoded by Abcb1) and breast cancer resistance protein (BCRP; encoded by Abcg2), separate the brain from the systemic circulation. The BBB responds to changes in microenvironment, such as inflammation, toxin exposure, or hormones. Extensive work in fetal sheep has shown that glucocorticoid exposure in utero increases expression and function of several TJ proteins, depending on time of exposure. Synthetic glucocorticoids (sGC) are administered to women at risk of preterm birth, to help drive fetal lung maturation. Changes in BBB function that persist after sGC exposure, could have ramifications for drug disposition in neonates. Currently, no studies have investigated whether fetal exposure to sGC leads to post-natal alterations in BBB integrity and function. OBJECTIVE: Determine the effect of antenatal sGC treatment on tight junction and drug transporter expression in microvessels of the juvenile BBB. METHODS: Guinea pigs were chosen for this study as they exhibit a similar pattern of neurodevelopment to humans, as well as similar developmental expression patterns for P-gp in the placenta and BBB. Guinea pigs were bred and treated with three courses of either vehicle or betamethasone (1 mg/kg) at gestation day (GD) 40, 41, GD 50, 51 and GD 60, 61. Brain endothelial cells were isolated and P-gp activity determined by Calcein-AM substrate accumulation assay. Brain microvessels were collected from offspring at postnatal day (PND) 14. Gene expression in isolated brain microvessels were analyzed by qRT-PCR. RESULTS: At PND14, sGC treatment resulted in decreased P-gp activity in cultured BECs in females but not males. Increased Cldn5 gene expression ($p<0.05$) was observed only in females, sGC treatment did not affect expression of drug transporters or other tight junction protein genes at PND14. CONCLUSION: Function of P-gp was decreased, and expression of Cldn-5, the main TJ protein, was increased, up to 24 days after sGC exposure, but only in females. Altered expression of TJs and drug transporters can lead to changes in BBB permeability. As such,

the sGC-induced changes observed could have profound implications on neonatal drug exposure, particularly in vulnerable preterm infants who are routinely exposed to multiple drugs. Supported by CIHR.

O11

Precise calculations of Iba-1 positive cells in cerebral hemispheres of mice

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Cutting tissues into thin slices has been the standard practice for many years in scientific research. This method provides two-dimensional information about the tissue. However, life occurs in three dimensions (3D); for this reason, scientists have always tried to extend tissue imaging to thick specimens. In neuroscience, visualization of intact brains in 3D is of intense focus. Previously, we developed a new imaging platform termed DIPCO (Diagnosing Immunolabeled Paraffin-Embedded Cleared Organs) that uses 3D light-sheet microscopy and whole-mount immunolabelling of cleared samples to study proteins and micro-anatomies deep inside of tumors (Tanaka et al., Nature Biomedical Engineering, 2017). Here, we have further optimized this method for whole-mount immunostaining of mice brains to calculate the number of cells in the intact tissue. We were able to accurately calculate the number of microglial cells to 1.599.622 in the cerebral hemisphere of a P28 mouse using the Iba-1 marker. To our knowledge this is the first time that the exact number of cells has been determined in a mouse brain. We believe that this pipeline can be applied for precise calculations of the cellular composition of various brain regions to help us understand the structure and function of the both developing and adult brain.

O12

The Role of Choline in Gestational Diets on Hypothalamic Food Intake Regulation in Wistar Rat Offspring

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Background: High gestational multivitamin diets induce an obesogenic phenotype in Wistar rat offspring, and folic acid (FA) contributes to these effects. However, imbalances between FA and other methyl-nutrients may exacerbate the observed outcome. Like FA, choline contributes to DNA methylation and may affect fetal brain development and later-life phenotype. The aim of this study is to elucidate the interaction between maternal choline and FA on in utero programming of hypothalamic food intake pathways and the associated later-life phenotypic outcomes.

Methods: Pregnant Wistar rats received an AIN93G diet containing either recommended choline (RC, 1 g/kg diet), low choline (LC, 0.5-fold), or high choline (HC, 2.5-fold) (Experiment 1). At birth, brain and blood was collected and at weaning, one male pup/dam was maintained on the RC diet for 17-weeks then terminated. Dependent measures include post-weaning FI, energy expenditure, body weight-gain, brain choline status, protein and gene expression of hypothalamic FI neurons, and gene-specific DNA methylation. In Experiment

2, pregnant rats will receive an AIN93G diet with either 1-fold FA and choline, or 5-fold FA with 0.5, 1.0, or 2.5-fold choline and will follow a similar protocol as Experiment 1.

Preliminary Results (Experiment 1): Brain choline concentrations at birth reflected the maternal diet content. At birth, HC pups had higher protein expression of the anorexigenic neuron pro-opiomelanocortin than both groups ($p<0.01$). Moreover, HC pups had higher expression of the orexigenic neuron neuropeptide-Y than LC pups ($p<0.05$), but not RC. During adulthood, offspring of HC dams had higher FI compared to RC ($p<0.01$), and higher weight-gain than RC and LC groups ($p<0.05$). Moreover, LC offspring had lower energy expenditure than both groups ($p<0.05$).

Conclusion: Maternal choline content may program FI and the relationship between body weight and energy expenditure in the offspring. The mechanisms underlying these novel findings are being investigated.

O13

Synthetic glucocorticoids during pregnancy and epigenetic outcomes in the brain and blood of offspring in a guinea pig model

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Synthetic glucocorticoids (sGC) are administered to pregnant women at risk of preterm delivery, approximately 10% of all pregnancies. Studies in animal models have shown that offspring exposed to elevated glucocorticoids, either by administration of sGC or endogenous glucocorticoids as a result of maternal stress are at increased risk of developing behavioral, endocrine, and metabolic dysregulation. DNA methylation has been proposed to play a critical role in long-lasting programming of gene regulation that underlie behavioural and physiological phenotypes in offspring. Peripheral tissues such as blood are an accessible source of DNA for epigenetic analyses in humans. Here we examined the hypothesis that prenatal sGC administration alters DNA methylation signatures in offspring hippocampus and whole blood. We compared these signatures across the two tissue types to assess epigenetic biomarkers potentially predictive of common underlying molecular pathways affected by sGC exposure. Pregnant guinea pigs were treated with sGC in late gestation and DNA methylation in approximately 3.7 million CpG sites was analyzed using reduced representation bisulfite sequencing in the hippocampus and whole blood from juvenile female offspring. Results indicate that there are tissue-specific methylation signatures of prenatal sGC exposure. Furthermore, among differentially methylated genes, 179 genes are modified in both brain and blood. Gene pathway analyses of these genes show that prenatal sGC exposure alters methylation of gene clusters involved in brain development and cardiovascular functions. These data may indicate a coordinated epigenetic programming in response to alterations in glucocorticoid signaling.

O14

The effects of folate dose and form during pregnancy on central and peripheral energy balance systems in Wistar rat dams and offspring

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Pregnancy is characterized by changes in maternal metabolism and although is "re-set" to homeostasis following birth, several factors may alter the homeostatic set point. Diet during pregnancy has long-term associated metabolic consequences, programming the mother and the offspring. In Wistar rat dams, high maternal multivitamin diets increase postpartum weight-gain to 16 weeks, and folic acid (FA) is a determinant of these effects. The objectives of my thesis are to examine whether this maternal programming is related to FA dose (Experiment 1) and/or folate form (FA vs bioactive folate, 5-methyltetrahydrofolate) (Experiment 2). Measures include postpartum phenotype, hepatic markers of folate metabolism, hypothalamic neuropeptides and peripheral regulators of energy metabolism in dams and female offspring.

Methods: In Experiment 1, pregnant Wistar rats were fed an AIN-93G diet containing 0X (low), 1X (recommended, 2mg FA/kg diet), 2.5X (moderate), 5X (high) or 10X (very high) levels of FA. Dams were fed the recommended diet during lactation and their first week post-weaning and then terminated.

Preliminary Results: The diets consumed during pregnancy affected the dams at four weeks after giving birth. Dams fed low (0X), and high (5X and 10X) FA during pregnancy had lower hepatic mRNA expression of key-methyl metabolism genes and protein compared to control suggestive of dysregulation of one-carbon pathways. Dams fed 0X- and 5X-FA also showed higher plasma insulin ($p<0.01$) and HOMA-IR ($p<0.05$) than dams fed the control diet, consistent with lower Glut4 and changes in mRNA of peroxisome-proliferator activated receptors in peripheral tissues. Body weight, food intake and hypothalamic food intake neurons were not affected by the maternal diets.

Preliminary Conclusion: Both low and high FA diets consumed during pregnancy left an imprint on methyl-metabolism genes and insulin regulation in the dams to four weeks post-birth, which may explain the previously observed long-term adverse effects of high FA maternal diets.

ABSTRACTS

Posters

P1
Molecular characterizaton of transcription-associated DNA double-strand breaks in neurodevelopment

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Transient DNA double-strand breaks (DSBs) have been implicated in transcriptional regulation of a subset of genes associated with neuroplasticity and development. In addition to their potential role in neurotypical brain development, DSBs have also been related to loss of genomic integrity and somatic mosaicism. To understand the role of DSBs in neurodevelopment, it is important to identify the transcription-associated DSBs and investigate the long-term effects of DSBs on genome integrity. In this project, the landscape of genome-wide DSBs will be assessed using Breaks Labeling In Situ and Sequencing (BLISS) in an iPS cell-based model of neurodevelopment. Changes in RNA-seq profiles and RNA synthesis will be correlated to the landscape of genomic DSBs across differentiation and in neuronal subpopulations. Altogether, this approach will allow us to formulate a list of candidate genes that are suspected to be integral in neurodevelopmental and neurodegenerative disorders. Finally, the long-term effects of repeated exposure to DSBs is likely to result in promotor mutagenesis and ultimately in structural genomic variation as observed in the human brain. This study aims to connect DSBs to genomic alterations observed in the brain and has the potential to elucidate new aspects of age-associated incidence of neurological disease.

P2
Regulation of Transport Across the Human Blood-Brain Barrier

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The developing mammalian brain is extremely vulnerable to environmental factors. Using animal models and primary cell culture, our group demonstrated that blood-brain barrier (BBB) transporter function is regulated by infection-mimic models and pro-inflammatory cytokines exposure, which induce the BBB breakdown, while exposure to glucocorticoids and growth factors up-regulate drug transporters function, indicating an increased fetal protection against potential xenobiotic factors. In this study, we hypothesize that environmental factors, such pro-inflammatory cytokines and growth factors, are important modulators of human developing BBB transporter function and that this regulation is mediated by fundamental

mechanisms including small non-coding RNAs and natural hormones. To address this hypothesis, human fetal brain samples from midgestation terminations will be obtained at Mount Sinai Hospital, and BECs and AST will be isolated and cultured, for posterior exposure to environmental factors and functional assessment. Immortalized human cerebral microvascular endothelial cell line (hCMEC/D3) is being used to optimize the protocols and It will be used for further comparison. This study will lead to improved understanding of the mechanisms that regulate the protection of the human fetal brain at the level of transporter function, allowing the development of strategies to improve protection of the fetal brain from potentially toxic environmental factors in normal and in compromised pregnancies.

P3

Differential DNA Methylation Signatures Following Prenatal Exposure to Single-course Antenatal Glucocorticoids in Humans and Guinea Pigs

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Background: Epidemiological studies have demonstrated associations between multiple antenatal corticosteroid (ACS) administration and neurocognitive and neurosensory impairments at 5yrs in children born at term. ACS is a standard clinical procedure provided to women presenting with a risk of preterm birth, to promote fetal lung maturation and reduce incidence of neonatal respiratory distress syndrome. Recently, reduction of ACS administration to a single course has been shown to have comparable neonatal benefits and reduced neurodevelopmental adversities. Similarly, guinea pig models of prenatal synthetic glucocorticoid (sGC) administration have also resulted in behavioural differences in exposed offspring. Our lab and others have reported differential epigenetic signatures following multiple-course ACS administration in humans and guinea pigs as a potential mechanism that underlies behavioural alterations. Human data are restricted to examination of peripheral tissues such as the placenta, umbilical cord or systemic blood, limiting our understanding of the direct effects of ACS on the developing brain. Conversely, we have identified region-specific DNA methylation signatures in guinea pig brains following sGC exposure in a sex-dependent manner, but it is unknown if epigenetic signatures in peripheral tissue are reflective of signatures in the brain. Hypothesis: It is hypothesized that ACS administration will establish distinct DNA methylation signatures in peripheral blood, which are comparable to signatures in the brain. Through this study, we aim to understand the relationship between DNA methylation signatures in the blood and the brain to delineate the impact of glucocorticoids in inducing epigenetic alterations across various tissues. Methods: Pregnant women who received antenatal corticosteroids between 24-34 weeks gestation at Sinai Health Complex were recruited with matched controls as part of the Ontario Birth Study (OBS). DNA from treated offspring was collected from neonatal heel prick blood spots 24h post-natal, to identify DNA methylation by bisulfite conversion. Converted DNA will be sequenced for analysis of global DNA methylation patterns. Similarly, pregnant guinea pigs were administered sGC or saline on GD50/51 and offspring brain and peripheral tissue were collected on post-natal day (PND)14. Tissues were rapidly frozen on dry-ice and are stored in -80˚C for DNA methylation analysis. DNA methylation and gene analyses of guinea pig tissues will be performed for comparative examination of signatures between peripheral blood and brain. Significance: Data from guinea pig will be highly valuable in understanding glucocorticoid-mediated mechanisms in human neurodevelopment.

P4

Pbx3: a novel regulatory gene during neuronal diversification in the developing enteric nervous system

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Within the wall of the gastrointestinal tract lies an intricate neuronal network, called enteric nervous system (ENS), responsible for the digestion of our daily food intake by regulating peristalsis, blood flow and secretion in the gut. It can comply with these functions due to its organization into a full reflex circuit consisting of sensory neurons, interneurons and motor neurons. The loss of neurons in the gut leads to life-threatening conditions such as Hirschsprung disease, in which the distal part of the colon is aganglionic. But also other diseases, such as achalasia, in which certain subtypes of neurons are selectively lost, can be found within the ENS. Despite the critical role of distinct enteric neurons for normal gut function, little is known about their molecular identities and subtype specification during the development. We have previously performed a temporal transcriptome analysis of lineage-traced enteric populations to identify transcription factors with potential regulatory functions during neuronal subtype diversification. Here we show a detailed expression pattern analysis of the transcription factor PBX3 in mouse and human developing gut tissue. Subsequent loss of function experiments in *Pbx3*$^{-/-}$ animals identified a deregulation of specific neuronal subtypes. We are right now setting up an over-expression system for *Pbx3* by utilizing ultrasound-guided nanoinjections into the developing mouse embryo. Furthermore we have established an iPS differentiation protocol for the generation of induced enteric neurons. We will be analyzing if over-expression of *Pbx3* will guide these cultures towards a specific neuronal fate.

P5

Anand Lakhani

P6

The effect of two novel mutations on DNA binding by bacterial integration host factor, sIHF

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P6

The Effects of Viral Mimics on Multi-drug Resistance in the Blood-brain Barrier

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INTRODUCTION: The blood-brain barrier (BBB), formed by brain endothelial cells (BECs), expresses P-glycoprotein (P-gp), a drug efflux transporter which functions to limit the passage of xenobiotics from the systemic circulation into the brain. The BECs are ensheathed by astrocytes (ASTs) which contribute to the immune response and increase P-gp activity within BECs. Early life viral exposure and resulting inflammation can deregulate BBB function allowing xenobiotics present within the systemic circulation into the brain. Dysregulated BBB has been linked to neurodevelopmental disorders. METHODS: The double-stranded RNA viral mimic Polyinosinic:polycytidylic acid (Poly (I:C)) and the single-stranded RNA viral mimic ssRNA40 were exposed to PND14 guinea pig AST mono-cultures for 24 hours at 10 and 25 ug/mL, and 2.5 ug/mL respectively. Quantitative reverse-transcriptase PCR was done to examine the expression of the pro-inflammatory cytokines IL-6, IL-1β, TNF-α and the toll-like receptors (TLR) 3/7/8. To determine the effect of viral mimics on P-gp activity a calcein-AM assay was undertaken after 4 and 24 hours Poly (I:C) exposure and 24 hours ssRNA40 exposure to PND14 BEC mono-cultures and AST/BEC co-cultures. RESULTS: ASTs had a significant increase in IL-1β mRNA after 24 hours ssRNA40 ($p<0.05$). IL-6 after Poly (I:C) and ssRNA40 and IL-1β after Poly (I:C) trended towards an increase. There were no changes in TLR 3 and 8 expression. TLR7 and TNF-α was not expressed within ASTs. There was no change in P-gp activity after Poly (I:C) and ssRNA40 exposure. CONCLUSIONS: Poly (I:C) and ssRNA40 induce a modest pro-inflammatory response within PND14 ASTs, however they do not alter P-gp activity. Future studies will attempt to understand the mechanism behind this response.

P7

Pluripotency transition and X-chromosome inactivation dynamics in pregastrula development revealed by single-cell RNA sequencing

Shangli Cheng, **Yu Pei,** Liqun He, Marlene Yilmaz, Björn Reinius, Qiaolin Deng*

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Characterization of in vivo counterparts of embryonic stem cells (ESCs) lays the foundation to manipulate them in vitro. However, the molecular features of the transition fromnaïve to primed epiblast (EPI) have remain unclear. Here, we performed single-cell transcriptomic analyses of the mouse pregastrula development during which the EPI undergoes the pluripotency transition and is primed for gastrulation. Our study revealed distinct molecular features of three cellular states in the EPI cells with increased expression of mesodermal signature genes through the transition state. Such dynamic process in vivo paralleled the continuum of naïve to primed ESCs transition in vitro. Moreover, we characterized the lineage-specific dynamics of X-chromosome inactivation(XCI). Intriguingly, X-chromosome reactivation (XCR) preceded completion of imprinted XCI silencing in the EPI, and the ensuing random XCI was asynchronous. This work provides new insights into the pluripotent EPI progression and XCI dynamics, and offers a detailed molecular roadmap to understand pluripotency dynamics.

P8

Selective Progesterone Receptor Modulators: Novel Therapeutic for Preterm Birth Prevention

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Pre-term birth is a chronic cause of perinatal mortality and developmental delays world-wide. Our preliminary findings have shown that progesterone receptor A isoform (PRA) becomes unliganded with the onset of labour which results in contraction associated protein expression, for example Connexin 43, becoming elevated. This trigger for parturition occurs there is nuclear progesterone withdrawal within the myometrium due to catabolism by the enzyme, 20□- hydroxyprogesterone dehydrogenase or AKR1C. All current interventions to prevent pre-term labour show limited efficiency therefore the development of a therapy is essential. Our current study is investigating if synthetic selective progesterone receptor modulator (SPRMs) could be the therapy to control myometrial contractions and prevent pre-term labour. SPRMs have the properties of binding to the progesterone ligand-binding pocket with the same affinity as progesterone and not being catabolized by 20□- hydroxyprogesterone dehydrogenase. If the progestin's properties can be used as a therapeutic during labour it will be able to ensure the liganded state, nuclear accumulation of PRB and repression of labour gene transcription persists in a quiescent state similar to progesterone before onset of labour. An in-vitro model will be used to identify SPRMs that mimic pregnancy progesterone conditions without the catabolism of the enzyme. This will be done by investigating the differences between the activity of progesterone and SPRMs in the context of binding, localization and labour gene protein expression levels. Progestogen therapy as a treatment to pre-term labour has shown promise in reducing the frequency of uterine contractions and reducing the rate of preterm deliveries and perinatal deaths. This data will provide further information for the progesterone receptor activity of SPRM candidates in the presence of an over-expressed AKR1C1 environment.

P9

Dynamics of histone variants in mESCs resolved by rapid amber suppressioncontrolled protein pulses

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As eukaryotic cells grow, proliferate and differentiate, modifications to their chromatin landscape occur in a dynamic manner. Many of those modifications are epigenetic, meaning that they are inherited through cell divisions. One of the epigenetic modifications that alter the structure of chromatin is the incorporation and eviction of histone variants in specific regions of the chromosomes. In my PhD project, focus is laid on further elucidating the dynamics of histone variants in stem cells, especially dissecting their cell cycle mediated crosstalk with interaction partners and their effect on chromatin.

For this, I use engineered mouse embryonic stem cells (mESCs) carrying an Amber Suppression machinery to rapidly express tagged histone variants and histone chaperones in presence of unnatural amino acids with various functionalities. This allows for a fast translation-mediated expression, allowing for resolving dynamics of histones with a fast time response of minutes to hours.

The main research questions are based around histone H3 and H2A variants. The centromeric specific histone H3 variant, CENP-A, is incorporated to chromatin during G1 phase and mislocalizes upon overexpression. Certain H2A variants have a characteristic role in X chromosome inactivation, where they will reside predominantly on the active or the inactive X chromosome. The research questions regarding the incorporation of the panel of histone variants to chromatin is being dissected using fluorescence microscopy and chromatin immunoprecipitation.

P10
Functional contribution of TGFß2 signalling to metanephric precursors and stromal cells

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Intrinsic ureteropelvic junction obstruction is the most common cause of congenital hydronephrosis, which affects approximately 1% of fetuses. Constitutive activation of Hedgehog signalling due to a Ptch1 deficiency in murine intermediate mesoderm–derived renal progenitors leads to hydronephrosis. Lineage tracing experiments indicate that Ptch1 deficient renal precursors give rise to an expanded stromal compartment as well as ectopically located stromal cells within the ureteropelvic junction causing blockage of urinary outflow and dilation of the renal pelvis. However, the underlying mechanism responsible for this altered specification remains unknown. We aim to define molecular mechanisms that underlie the effects of Hedgehog signalling on stromal cell specification from their uncommitted precursor population. Preliminary evidence implicates transformation growth factor beta 2 (TGFß2), a secreted factor expressed within the stroma, as one possible downstream effector of Hedgehog signalling. Histological analysis of kidneys in which Tgfbr2, encoding TGFß receptor II, is deleted in the renal progenitors will further reveal the role of TGFß signalling in this lineage. We will also remove Tgfbr2 in renal progenitors with a Ptch1 null genetic background to investigate if the lack of TGFß signalling rescues the abnormalities that arise from Ptch1 deficiency. Together, these experiments will help elucidate downstream mechanisms of Hedgehog signalling in normal and malformed kidneys as well as identify potential therapeutic targets.

P11
Marine Compound Xyloketal B Reduces Neonatal Hypoxic-Ischemic Brain Injury

Ai-Jiao Xiao[1,†], Wenliang Chen[1,2,3,†], Baofeng Xu[1,2,†], Rui Liu[1], Ekaterina Turlova[1,2], Andrew Barszczyk[1], Christopher LF Sun[1], Ling Liu[1], Marielle Deurloo[1], Guan-Lei Wang[4], Zhong-Ping Feng[1,*] and Hong-Shuo Sun[1,2,3,5,*]

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Neonatal hypoxic-ischemic encephalopathy causes neurodegene -ration and brain injury, leading to sensorimotor dysfunction. Xyloketal B is a novel marine compound isolated from a mangrove fungus Xylaria species (no. 2508) with unique antioxidant effects. In this study, we investigated the effects and mechanism of xyloketal B on oxygen-glucose deprivation-induced neuronal cell death in mouse primary cortical culture and on hypoxic-ischemic brain injury in neonatal mice *in vivo*. We found that xyloketal B reduced anoxia-induced neuronal cell death *in vitro*, as well as infarct volume in neonatal hypoxic-ischemic brain injury model *in vivo*. Furthermore, xyloketal B improved functional behavioral recovery of the animals following

hypoxic-ischemic insult. In addition, xyloketal B significantly decreased calcium entry, reduced the number of TUNEL-positive cells, reduced the levels of cleaved caspase-3 and Bax proteins, and increased the level of Bcl-2 protein after the hypoxic-ischemic injury. Our findings indicate that xyloketal B is effective in models of hypoxia-ischemia and thus has potential as a treatment for hypoxic-ischemic brain injury.
Keywords: hypoxic-ischemic injury; infarct volume; neuroprotection; oxygen glucose deprivation; primary neuronal cell culture; neonatal stroke; behavioral tests; marine drug

P12

Developmental trajectories towards the oligodendrocyte lineage in the mouse and human brain: insights from single-cell RNA-Seq

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Oligodendrocytes (OLs), originating from the glial lineage are myelinating cells populating the CNS that facilitate electrical impulse transmission through axon ensheathment. In demyelinating diseases neurodegeneration occurs, particularly in multiple sclerosis (MS) where an immune response is mounted against myelin. Oligodendrocyte precursor cells (OPCs) are recruited to lesions and remyelinate axons, however, this mechanism fails in progressive MS. The oligodendrocyte lineage, from OPCs towards mature OLs, involves transitions along several states that define differentiation and myelination potential. In our work in mice, geared to reveal distinct epigenetic states during development, we have individually sequenced 6565 cells belonging to the oligodendrocyte lineage from the mouse brain with developmental time points ranging from embryonic day 13.5 to postnatal day 90. We identified several well-defined populations of oligodendrocyte lineage cells reflecting unique stages during the process of differentiation and myelination. Additionally we have single-cell expression data of more than 14000 single cells of human forebrain during development where we delineate the generation of the oligodendrocyte lineage in naive and differentiated states, derived from fetal samples between the ages of 8-10 weeks.

We have developed a combination of algorithms to deduce lineages from single-cell transcriptional data across an abstract manifold and use this to compare the pseudo-temporal expression patterns of regulators and non-coding RNAs between mouse and human early development to elucidate the developmentally conserved use of non-coding RNAs and DNA elements across early forebrain development, with a particular focus on the oligodendrocyte lineage.

P13

Preliminary Investigation Of The Utility Of Mri For Measuring The Hematocrit In Fetal Anemia

Jiawei Xu[1,5]; An Qi Duan2; Johannes Keunen3; Sharon Portnoy4; John Sled4; John Kingdom[3]; Christopher K. Macgowan[4]; Mike Seed[1,5]

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Plasmodium falciparum (Apicomplexa) uses complex strategies for survival, transmission and immune system evasion and is still a leading cause of human death. Important parasitic phenotypes, e.g. drug-induced quiescent cells, cell sequestration and sexual commitment, occur at low frequencies within parasitic populations and have therefore been hard to molecularly profile. Here, we have explored the potential of single-cell RNA-sequencing to profile individual and groups of malaria infected red blood cells (miRBCs) at different stages of the intra-erythrocytic life cycle. Analyses of parasite gene expression in single miRBCs identified known life cycle markers but also revealed considerable heterogeneity within miRBC picked at the same time point after culture synchronization. The miRBCs grouped into eight subpopulations by their expression of gene signatures important for its intra-erythrocytic life cycle. Importantly, we identified three miRBCs that had initiated sexual commitment and they specifically expressed a unique signature of genes that included many novel genes not previously associated with sexual commitments. This study highlights the exciting potential in using single-cell RNA-sequencing to profile rare and pathologically important sub-groups of parasites.

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Developmental and Perinatal Biology 2018

Course Evaluation

Please complete and RETURN the evaluation. This is **important** because your valuable input will help us design future courses. Thank you.

Trainee from: (please tick) Karolinska Institute _____ University of Toronto _____

Lecture Course: (please circle one)

1) Stem Cells, Embryonic Development and Disease

could be improved	1	2	3	4	5	*excellent*
a) Eszter Posfai	1	2	3	4	5	
b) Fredrik Lanner	1	2	3	4	5	
c) Cristina Nostro	1	2	3	4	5	
d) Michael Laflamme	1	2	3	4	5	

2) Placenta & Birth:

could be improved	1	2	3	4	5	*excellent*
a) Brian Cox	1	2	3	4	5	
b) John Sled	1	2	3	4	5	
c) Lubna Nadeem:	1	2	3	4	5	
d) Isabella Caniggia	1	2	3	4	5	

3) Lung/Heart Development:

could be improved	1	2	3	4	5	*excellent*
a) Tom Waddell	1	2	3	4	5	
b) Brian Kavanagh	1	2	3	4	5	
c) Ronald Li	1	2	3	4	5	
d) P. Delgado Olguin	1	2	3	4	5	

4) Neurodevelopment:

could be improved	1	2	3	4	5	*excellent*
a) Kristiina Tammimies	1	2	3	4	5	
b) Julie Lefevbre	1	2	3	4	5	
c) Jim Eubanks	1	2	3	4	5	
d) Amy Ramsey	1	2	3	4	5	

5) Developmental Origins of Health and Disease:

could be improved	1	2	3	4	5	*excellent*
a) Anna Wredenberg	1	2	3	4	5	
b) Ola Hermanson	1	2	3	4	5	
c) Stephen Matthews	1	2	3	4	5	
d) Patrick McGowan	1	2	3	4	5	

Please outline how you feel the lecture course could be improved?

Practical Courses:

Please indicate which 3 practical courses you attended and evaluate each below:

1. Generation of genetically modified mouse models ☐
2. Introduction to R and graphics for biology ☐
3. NICU Visit at Mount Sinai Hospital ☐
4. Centre for Commercialization of Regenerative Medicine (CCRM) ☐
5. Tissue Biobanking in Reproductive Biology ☐
6. Methods and Tools to Assess Embryo Quality in the IVF clinic ☐

1. Generation of genetically modified mouse models

Course Content:

could be improved 1 2 3 4 5 *excellent*

Course Organization:

1 2 3 4 5

2. Introduction to R and graphics for biology

Course Content:

could be improved 1 2 3 4 5 *excellent*

Course Organization:

1 2 3 4 5

3. NICU Visit at Mount Sinai Hospital

Course Content:

could be improved 1 2 3 4 5 *excellent*

Course Organization:

1 2 3 4 5

4. Centre for Commercialization of Regenerative Medicine (CCRM)

Course Content:

could be improved 1 2 3 4 5 *excellent*

Course Organization:

1 2 3 4 5

5. Tissue Biobanking in Reproductive Biology

Course Content:

could be improved 1 2 3 4 5 *excellent*

Course Organization:

1 2 3 4 5

6. Methods and Tools to Assess Embryo Quality in the IVF clinic

Course Content:

could be improved 1 2 3 4 5 *excellent*

Course Organization:

How could the practical courses be improved?

Thank You