Southern Ontario Reproductive Biology Conference

Queen's University School of Medicine

Wednesday May 6th, 2026

Organizing Committee

Dr. David Natale & Dr. Tiziana Cotechini
Ruslan Amruddin
Bryony Natale
Florella Peng

Queen's University

SORB logo designed by Melanie Lemaire

Land Acknowledgement

Queen's University is situated on the traditional territory of the Anishinaabe and Haudenosaunee peoples. These lands and waters continue to be home to Indigenous communities who maintain long-standing relationships with this place. We are grateful for the opportunity to gather, learn, and work here.

This meeting focuses on reproductive biology, a field concerned with the conditions that allow life to begin, develop, and persist across generations. Our research is inseparable from the land, water, and ecological systems that sustain life. We acknowledge that our ability to gather and conduct this work is grounded in the long stewardship of Indigenous peoples, and we recognize our responsibility to approach our research with care, integrity, and respect for future generations.

A Short History of the Southern Ontario Reproductive Biology (SORB) Meeting

As we embark on the 53rd Annual SORB conference today, it is important to reflect on its history. This meeting emerged as a grassroots, community-driven gathering of reproductive biology researchers across Southern Ontario. It developed in response to a shared need for a local, accessible forum where scientists could present ongoing work, exchange ideas, and foster collaboration without the barriers associated with large national or international conferences.

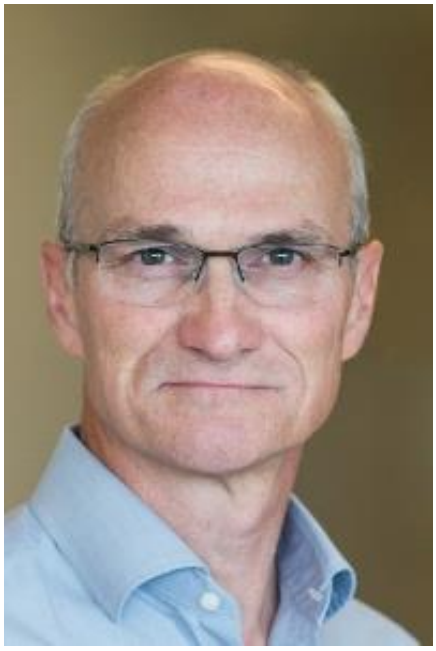
From its early years, SORB has been characterized by its rotating host model, with universities and research institutions across Southern Ontario taking turns organizing the meeting. This structure helped build a strong regional network spanning basic, translational, and clinical reproductive science, including faculty, trainees, and clinician-scientists. A defining feature of SORB has always been its strong emphasis on trainees. Graduate students, postdoctoral fellows, and early-career researchers are central to the program, with opportunities to give oral and poster presentations in a supportive environment. This focus has made SORB an important training ground for developing scientific communication skills and interdisciplinary thinking within reproductive biology.

Over time, the scope of SORB has evolved alongside the field itself, expanding to include topics such as placental biology, developmental origins of health and disease, reproductive toxicology, fertility, endocrinology, and maternal–fetal health. Despite this growth, the meeting has intentionally remained small and collegial, typically drawing around 100 participants, which preserves open discussion and accessibility. Today, SORB continues to serve as a regional anchor for reproductive science in Southern Ontario, valued for its collaborative spirit, commitment to mentorship, and role in strengthening connections across institutions and disciplines. Its longevity reflects the community's shared commitment to maintaining a meeting that prioritizes scientific rigour, inclusivity, and the next generation of reproductive researchers.

Thank you for joining us,

Dave and Tiziana
Co-Organizers, SORB-53rd Annual Conference

Keynote Speaker



Dr. Graeme N. Smith, MD, PhD, FRCSC, FCAHS
Professor Emeritus, Department of Obstetrics and Gynaecology
Queen's University

"*Pregnancy Complications and Future Cardiovascular Disease*"
1:00 – 1:30 pm

Dr. Graeme N. Smith is a clinician-scientist whose career demonstrates the integration of reproductive biology, clinical research, and training the next generation of scientists and clinicians. He is Professor Emeritus in the Department of Obstetrics and Gynaecology at Queen's University and the founding Director of the Queen's Perinatal Research Unit, a program that combines basic science, clinical investigation, and population-level research.

Dr. Smith's work has focused on pregnancy as a critical window into lifelong health, particularly how adverse pregnancy outcomes—such as pre-eclampsia—inform future maternal cardiovascular and metabolic risk. His research has contributed to reframing reproductive biology as foundational to long-term health, highlighting the translational impact of reproductive science on patient care and public health.

Trained through a combined MD/PhD program at the University of Western Ontario, followed by specialty training at Queen's University and subspecialty training in Maternal-Fetal Medicine at the University of Toronto, Dr. Smith joined Queen's faculty in 1999. He served as Head of the Department of Obstetrics and Gynaecology from 2013 to 2023 while maintaining an active clinical practice in high-risk obstetrics.

A committed mentor and educator, Dr. Smith has supervised over 100 graduate students and clinical research trainees and has authored more than 300 peer-reviewed publications. He developed the MotHERS Program (Mothers' Health through Education, Research, and Screening) to improve maternal health outcomes and has received numerous honours recognizing the international impact of his work, including Fellowship in the Canadian Academy of Health Sciences, a King Charles III Coronation medal and the CNPRM Grand Voyageur Award.

Dr. Smith's career reflects the diverse paths available in reproductive sciences—from bench research to clinical translation—and underscores the impact trainees can have when scientific discovery is paired with mentorship and collaboration.

SORB 2026 Program:
Queen's University, School of Medicine rm 132

8:30 – 9:15: Registration, Poster Set-up & Continental Breakfast

9:15 – 9:30: Welcome

9:30 -10:45: Oral Presentations I – *Chair: Dr. David Natale*

9:30-9:45 Endometriosis patient-derived extracellular vesicles exhibit stage- and tissue-specific signatures and functional effects in vitro
Jaelis P Holmes, Queen's University

9:45-10:00 Interleukin-33-Activated Regulatory T Cells in Endometriosis
Ola Wodz, Queen's University

10:00-10:15 Pancreatic Beta-cell Plasticity: Trans-differentiation of Delta- to Beta-cells during pregnancy
Bavina Thirunavukarasu, Western University

10:15-10:30 Impacts of a Maternal Obesogenic Diet on Uteroplacental Fibrin Deposition in the Mouse
Sophie Johnston, McMaster University

10:30-10:45 Prenatal Phytocannabinoid Exposure Reprograms Pancreatic Alpha and Beta Cell Function in Adult Offspring
Samuel Ugulini, Western University

10:45 – 11:00: Break – Coffee/Tea/Snacks

11:00 – 12:00: Oral Presentations II and Flash Talks – *Chair: Dr. Maha Othman*

11:00-11:15 Seminal Plasma BPS levels correlate to AMH signalling and semen parameters in IVF patients
Nathan Galvao, University of Guelph

11:15-11:30 miR-to-miR interactions: BPA and its analogs dysregulate key miRs and developmental parameters in a miR-21 independent manner in COCs and in vitro produced embryos
Fiona Mcilhargey-Larkin, University of Guelph

11:30-11:45 Protein Dysregulation in Pre-eclampsia: Precision Peptide-Based Molecular Insights
Thomas Kazmirchuk, Queen's University

11:45-12:00 **FLASH TALKS**

- Accelerating Aptamer Discovery for Bovine Sperm Sexing using a Data-driven Non-Iterative SELEX Framework
  Nikita Gahoi, University of Waterloo
- Triphenyl phosphate disrupts estrogenic, glycolytic and lipid homeostasis: integrated meta-analysis across five vertebrate species and functional validation in aquatic embryonic cells
  Holly Mackay, Queen's University
- Platelet GPIbα Dysfunction Is Associated with Abnormal Placental Structure and Adverse Pregnancy Outcomes
  Vrisha Shah, Queen's University
- Enhancing Early Obstetric Ultrasound Competency in Medical Education: A Resident-Led POCUS Workshop Evaluation
  Fariya Zaheer, Queen's University

12:00 – 1:00: Lunch

1:00 – 1:30: Keynote Presentation – Dr. Graeme Smith, Queen's University
"*Pregnancy Complications and Future Cardiovascular Disease"*

1:30 – 3:00: Poster Session – SOM Atrium

- Even numbers 1:30 – 2:15
- Odd numbers 2:15 – 3:00

3:00 – 3:45: Oral Presentations III – *Chair: Dr. Steve Renaud*

3:00-3:15 Cyclosporin A treatment rescues mitochondrial apoptotic marker expression in a hypoxia-induced syncytiotrophoblast stress model of preeclampsia
Jenny Feeny, McMaster University

3:15-3:30 OVOL2 Reinforces Epithelial Identity and Mediates Syncytiotrophoblast Formation in the Mouse Placenta
Violet Patterson, Western University

3:30-3:45 Fluoxetine-Induced Oxidative Stress Disrupts Tryptophan and VEGFA Signaling in Human Placenta Cells
Rodrigo Vargas, McMaster University

3:45 – 4:15: Break - Refreshments

4:15 – END: AWARDS and Farewell

Code of Conduct

This meeting is committed to providing a respectful, inclusive, and professional environment for all participants.

Photography and Recording Policy

To foster open scientific exchange and protect unpublished work:

- Photography, audio recording, or video recording of presentations, posters, or discussions is not permitted unless explicit permission is granted by the presenter.
- Screenshots or recordings of virtual sessions are also prohibited without permission.
- Presenters may indicate on their slides or posters if photography is permitted.

Attendees are encouraged to respect these boundaries and ask presenters directly before capturing any images or recordings.

Data Confidentiality and Unpublished Data Guidance

This meeting includes the presentation of unpublished data and preliminary findings. By attending, participants agree to:

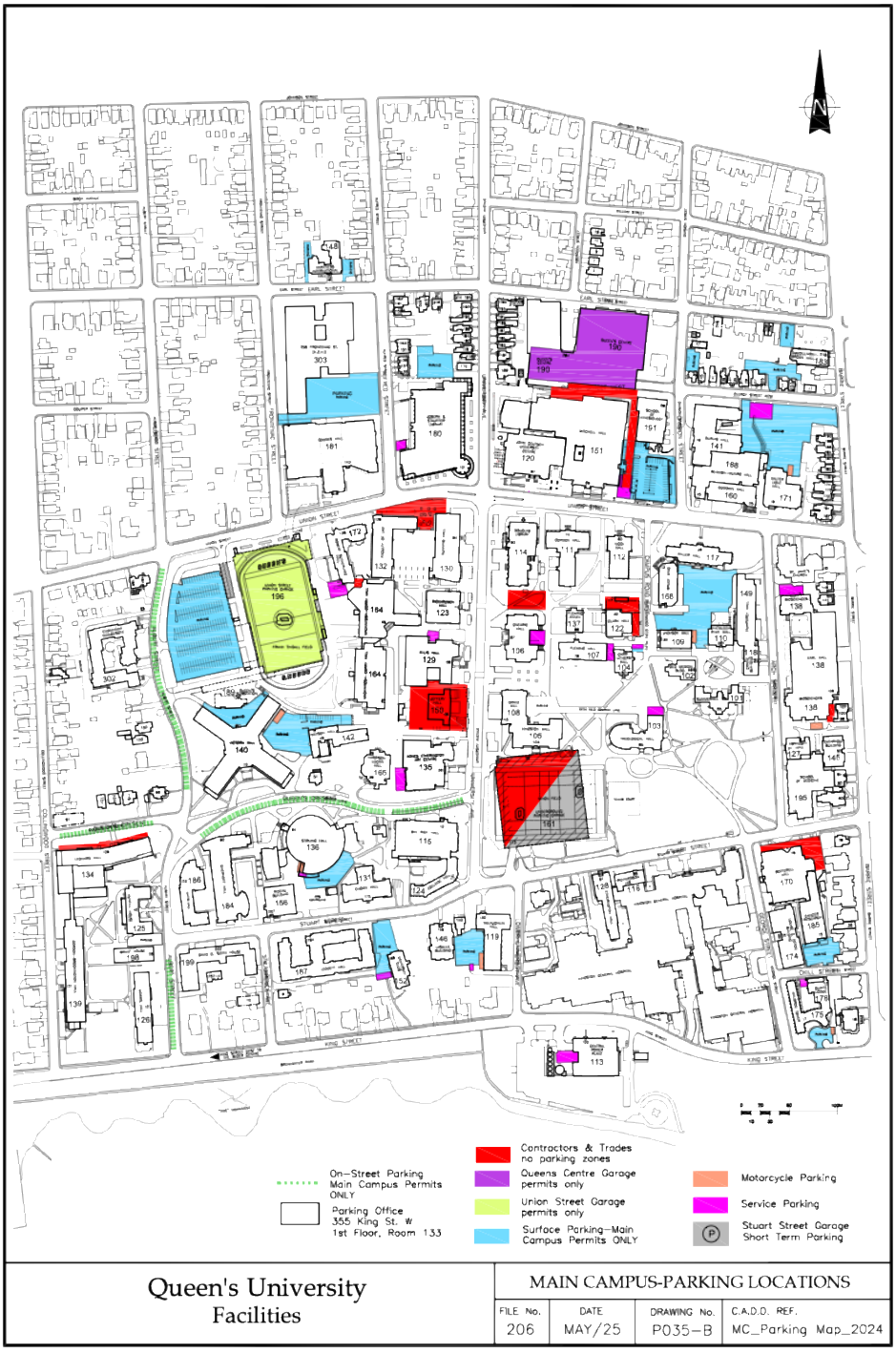
- Treat all presented data, ideas, and discussions as **confidential**
- Not share, distribute, or publicly discuss content presented at the meeting without the explicit permission of the presenter
- Refrain from using unpublished data presented at the meeting for personal research, grant applications, or publications without prior consent

These practices are especially important in a trainee-focused meeting and are intended to support open dialogue, collaboration, and trust within the reproductive research community.

Thank you for helping to create a collegial, respectful, and scientifically rigorous meeting environment.

Location and Parking Information

- The meeting will be held in the School of Medicine Building on Arch Street, which is outlined in a blue rectangle on the attached map
- **Parking passes will be available at the registration table**
- Participants may park in any of the light blue–shaded parking lots indicated on the map; the lots that are most easily accessed are circled in red.



Venue Accessibility Information

Building: New Medical Building
Address: 15 Arch Street /80 Barrie Street
Located south of Abramsky All and across from Botterell Hall

The New Medical Building is designed to support accessibility for all attendees. All floors include accessible washroom facilities. Single-user washrooms across campus are gender-neutral unless otherwise signed, in accordance with Queen's University's inclusivity policy. Elevators serving all floors are fully accessible and equipped with audible announcements and Braille buttons.

First Floor Washrooms

- One single-user accessible washroom with a power door, located near Room 106
- Single-user washrooms without power doors, located near Rooms 111 and 113
- Multi-user washrooms on this floor are accessible and do not have any exterior entry doors

Thank you to our sponsors:

Many thanks to our superstar helpers:

Conference Setup: Logan Bale, Phillip Stachera, Abbie Koshan, Emily Suggitt, Tony D'Alessio, George Ilbawi, Paige Turner, Joey Qiao and Lainie Spencer

Chairs: Dr. Maha Othman, Dr. Steve Renaud

Judges (Talks): Dr. Dean Betts, Dr. Tim Regnault, Dr. Rafael Sampaio

Judges (Posters): Dr. Charles Graham; Dr. Nikki Philbrook; Dr. Laura Favetta; Dr. Jean-Francois Pare; Zuleika Leung, MSc; Sarah Nash; Richard Nauman and Katie Zutautas

8:30 -9:15 Registration, Poster Set-up & Continental Breakfast

9:15 -9:30 Welcome

9:30 -10:45: Oral Presentations I – *Chair: Dr. David Natale*

OP1. Endometriosis patient-derived extracellular vesicles exhibit stage- and tissue-specific signatures and functional effects *in vitro*

Jaelis P. Holmes, Katherine B. Zutautas, Danielle J. Sisnett, Chandra Tayade

Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada

Endometriosis (EM) is a chronic inflammatory disorder characterized by the presence of endometrium-like tissue outside the uterine cavity, affecting ~10% of reproductive-age women. It is a leading cause of pelvic pain and infertility, with diagnosis often significantly delayed due to heterogeneous symptom presentation. While EM pathogenesis remains incompletely understood, small extracellular vesicles (sEVs) have emerged as potential mediators of disease progression and novel biomarkers. sEVs carry bioactive cargo and exhibit distinct molecular profiles in EM patients, with roles in modulating immune and inflammatory pathways. This study aimed to define disease stage- and tissue-specific sEV signatures and their contributions to EM pathophysiology.

sEVs from mild and severe EM were profiled across ectopic lesions, eutopic endometrium, plasma, peritoneal fluid, and healthy controls, using nanoparticle tracking analysis, transmission electron microscopy, and MACSPlex flow cytometry (39 surface markers). Surface marker analysis revealed distinct stage- and tissue-specific sEV signatures, including immune profile shifts in severe EM and differential expression of immune, adhesion, and stemness-associated markers linked to disease progression and lesion invasiveness.

Functional impact of EM patient-derived sEVs on key pathological processes were assessed in EM-representative cell lines (12Z, hESC, HUtMEC), with uptake confirmed across all cell types. Mild lesion-derived sEVs increased proliferation in endometrial epithelial (12Z) and endothelial (HUtMEC) cells, while severe lesion-derived sEVs enhanced endothelial tube formation in HUtMECs. Cytokine profiling further supported stage- and tissue-specific effects, with mild lesion-derived sEVs inducing elevated immune-associated cytokines and chemokines.

Collectively, EM-derived sEVs exert distinct stage-dependent functional effects *in vitro*, supporting roles in lesion establishment and progression and highlighting their potential as biomarkers in endometriosis. This work was supported by NSERC and CIHR.

OP2. Interleukin-33-Activated Regulatory T Cells in Endometriosis

Alexandra Wodz[1], Katherine B. Zutautas[1], Dan Vo Hoang[1], Chandrakant Tayade[1]

[1]*Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, CA.*

Background: Endometriosis (EM) is a chronic inflammatory disease affecting ~10% of women, characterized by the growth of ectopic (extra-uterine) endometrial-like lesions. Our lab has shown elevated interleukin-33 (IL-33) in peritoneal fluid (PF), plasma, and lesions of EM patients. IL-33, a Th2-associated cytokine, signals via the suppressor of tumorgenicity 2 (ST2) on immune cells including regulatory T cells (Tregs). Emerging studies implicate both IL-33ST2 axis and Tregs in inflammation and lesion growth, yet their relationship in EM remains undetermined. We hypothesize that IL-33-ST2 signalling drives Treg expansion and pathogenic reprogramming, promoting immune tolerance, inflammation, and lesion progression.

Methods: qRT-PCR was performed on EM lesions, eutopic, and control endometrium for Treg biology-related genes. *In vitro*, CD4+ murine splenocytes were activated (anti-CD3/CD28, IL-2) ± IL-33 (N=3/treatment) to assess Th1, Th2, and Treg frequencies. *In vivo*, C57BL/6 mice underwent EM or SHAM surgery, and later received i.p. injections of rmIL-33 or PBS (N=6/group) every other day for 6 doses. Flow cytometry characterized immune populations in the spleen, peritoneal fluid (PF), endometrium, and lesions. Cell culture supernatant and PF were characterized by multiplex cytokine array.

Results: qRT-PCR revealed elevated FOXP3, CD25, CCR5, and CCR6**.** *In vitro,* IL-33 treatment increased Th2 cell frequency, Th2-type cytokines, and Treg ST2 expression. IL-33 significantly enriched Treg frequencies *in vivo* in spleen and PF. PF cytokines revealed a Th2 skew. In EM conditions, IL-33 did not markedly Treg proportions but increased ST2 expression on Tregs in the spleen and PF.

Conclusion: IL-33 promotes a Th2-skewed environment in EM. It enhances Treg-associated markers and cytokines, and may contribute to immune tolerance and lesion progression, potentially though Treg-mediated mechanisms.

Funding: CIHR, NSERC.

OP3. Pancreatic Beta-cell Plasticity: Trans- differentiation of Delta- to Beta-cells during Pregnancy

Bavina Thirunavukarasu, Dawn Kumar, Edith Arany and David Hill

Background: The number of insulin (Ins)-secreting β-cells increases in the maternal pancreas during pregnancy to compensate for insulin resistance. This involves a reinitiation of β-cell proliferation, although transdifferentiation from other islet cell types, such as somatostatin (Sst)secreting δ-cells, may also occur. It is unclear whether δ-cell mass (DCM) is similarly increased during pregnancy, the extent to which δ- to β-cell transdifferentiation contributes to the increased β-cell mass (BCM), or how these might change in a diabetic pregnancy.

Methods: We used a transgenic mouse model to trace δ-cell lineage fate using a yellow fluorescent protein (YFP) genetic lineage tag. Pancreas sections were collected from nonpregnant (NP) females and from pregnant mice at gestational days (GD) 9, 12, 15, and 18, and processed for immunofluorescence histochemistry. Cells positive for Ins and YFP but negative for Sst ($Ins^{+}YFP^{+}Sst^{-}$) represented new δ-cell-derived β-cells. Mice received either no injection, buffer alone, or streptozotocin (STZ) to induce β-cell loss. Two weeks post-injection, mice were mated and pancreas removed for image analysis.

Results: Both BCM and DCM significantly increased by GD18 compared to NP. Delta to β-cell ratio was significantly greater by GD18. Lineage tracing showed a 5-6-fold increase in both bihormonal and transdifferentiated cells by GD18 in normal pregnancy, although transdifferentiated cells were 60% less abundant than bihormonal. Mice treated with STZ had an initial 40% reduction in BCM and were glucose intolerant during pregnancy. The adaptive increase in BCM, but not DCM, was impaired in diabetic pregnant mice, although the relative presence of bihormonal and δ-cell-derived β-cells was increased, relative to buffer-treated controls.

Conclusion: Both maternal BCM and DCM increased during pregnancy and increased δ to β-cell transdifferentiation occurred with a bihormonal intermediate phenotype. During diabetic pregnancy the increase in BCM was impaired, although the relative contribution of transdifferentiation to generate new β-cells was increased

OP4. Impacts of a Maternal Obesogenic Diet on Uteroplacental Fibrin Deposition in the mouse

Sophie Johnston, Ariana Lewis, Christian Bellissimo, Deborah Sloboda

Department of Biochemistry and Biomedical Sciences, McMaster University

Background and Objectives: We have shown that maternal excess adiposity is associated with chronic, low-grade inflammation, hypoxia and vascular dysfunction at the uteroplacental interface and contributes to adverse pregnancy outcomes. We hypothesized this hypoxia arises from dysregulation of coagulation pathways in these pregnancies, promoting excessive fibrin deposition and vascular obstruction. We aimed to identify spatial and temporal characteristics of decidual fibrin deposition across gestation and determine whether a maternal obesogenic diet alters these patterns.

Methods: Female C57BL/6J mice were assigned to either a control (3.0 kCal/g: 17% kcal fat, 54% kcal carbohydrate, 29% kcal protein) or high-fat, high-sucrose diet (4.73 kCal/g: 45% kcal fat, 17% kcal sucrose, 18% kcal carbohydrate, 20% kcal protein) for 12 weeks prior to mating with control fed males. Uteroplacental tissues were collected at embryonic days (E)12.5 (CON n=5, HFHS n=5) and E18.5 (CON n=4, HFHS n=6), fixed and processed for histological evaluation. Martius, Scarlet and Blue staining was used to identify fibrin deposition and distinguish newly deposited versus old fibrin. Decidual vessel occlusions were assessed qualitatively. Fibrin levels were evaluated using a trained pixel classifier in QuPath. Statistical analyses were conducted using two-way ANOVA with maternal diet and gestational timepoints as factors.

Results: We found that the number of fully occluded decidual blood vessels increased with gestation ($p = 0.0004$) and was elevated in obesogenic diet pregnancies, particularly at E18.5 ($p = 0.0083$). Gestational age was a primary driver of fibrin deposition, with increased accumulation of older fibrin and less newly deposited fibrin at E18.5 versus E12.5 ($p < 0.0001$). Decidual fibrin deposition was not impacted by maternal diet at either gestational timepoint. Fetal sex did not significantly affect fibrin deposition.

Conclusion: Maternal excess adiposity increased vascular occlusion late in gestation suggesting that obesity-associated coagulation disturbances may manifest within the uteroplacental vasculature rather than the decidual tissue itself. Decidual fibrin deposition increased with advancing gestational age, likely reflecting trophoblast remodelling, turnover and repair.

Funding: CIHR, Canada Research Chair Program

OP5. Prenatal Phytocannabinoid Exposure Reprograms Pancreatic Alpha and Beta Cell Function in Adult Offspring

Samuel Ugulini[1], Daniel Hardy[3,5], Savita Dhanvantari[1,2,4]

[1]Pathology and Laboratory Medicine, [2]Medical Biophysics, [3]Physiology and Pharmacology, Schulich School of Medicine & Dentistry, Western University, [4]Metabolism & Diabetes Imaging Program, Lawson Research Institute, [5]Children's Health Research Institute, London ON, Canada

Background: There is emerging evidence that prenatal cannabis exposure predisposes offspring to type 2 diabetes (T2D) later in life. Recent work from our group has shown that gestational exposure to Δ^9-tetrahydrocannabinol (Δ^9-THC) may alter glucose tolerance, insulin sensitivity and glucagon trafficking and secretion in adult rat offspring in a sex-specific manner. Therefore, we propose that

prenatal cannabis exposure negatively affects pancreatic islet development and function, leading to long-term and sex-specific metabolic consequences in adult offspring.

Methods: Pregnant Wistar rats received daily i.p. injections of vehicle or ∆-9 THC (3 mg/kg) from gestational days 6 – 22 and were sacrificed at 21 days (juvenile) and 5-months of age (adult). Pancreatic tissue was assessed for glucagon, insulin and Lamp2 expression via immuno-fluorescent confocal microscopy. Subcellular localization of fluorescent reporters was analyzed using plot profile analysis.

Results: In juvenile female offspring, Δ^9-THC-exposure resulted in significant increases in α-cell glucagon content and decreases in Lamp2 lysosomal trafficking in ß-cells (n=3, $p<0.05$). Strikingly, Lamp2 expression was significantly altered between juvenile, and adult female offspring, where Δ^9-THC-exposure caused a complete loss of Lamp2 lysosomal trafficking in both α- and ß-cells (n=3, $p<0.05$). No changes were observed in the male cohort.

Conclusion: Prenatal Δ^9-THC exposure disrupts the normal endocrine function of the pancreas, altering glucagon, insulin, and lysosomal trafficking in an age- and sex-specific manner. These consequences, which seem to manifest later-in-life alongside metabolic dysfunction, predispose the affected offspring to the development of T2D.

10:45 – 11:00: Break – Coffee/Tea/Snacks

11:00 – 12:00: Oral Presentations II and Flash Talks – *Chair: Dr. Maha Othman*

OP6. Seminal Plasma BPS levels correlate to AMH signalling and semen parameters in IVF patients

Galvao, NA.[1], Rutherford, SIJ.[1], Lagunov, A.[2], Neal, MS.[3,4], Favetta, LA.[1]

[1]Reproductive Health and Biotechnology Lab, OVC, University of Guelph, Guelph, ON, [2]CCRM/Hannam Fertility Clinic, Toronto, ON, [3]ONE Fertility, Burlington, ON, [4]Department of Obstetrics and Gynecology, McMaster University, Hamilton, ON.

Background: Bisphenols are plasticizers used in everyday products. Since BPA's ban in 2010, bisphenol S (BPS) is one of the most used analogues. BPS disrupts Anti-mullerian hormone (AMH) signalling in oocytes and embryos, but its effects are unknown in sperm. AMH is secreted by Sertoli cells during fetal development, but decreases at puberty, with no known function in adults. Seminal plasma AMH is correlated with sperm concentration and motility, and the AMH receptor (AMHRII) is expressed on mature sperm, supporting a physiological role.

Methods: Seminal BPS was measured by ELISAs in donated human semen. Samples were separated into low and high BPS groups (<300pg/mL=Low; >600pg/mL=High). RNA was extracted and AMHRII, SMAD1, and SMAD4 transcripts were measured by qPCR (n=10). AMH, AMHRII, SMAD1, phosphorylated-SMAD1 (P-SMAD1) and SMAD4 proteins were localized by Confocal Microscopy and quantified by ImageJ software. Known semen parameters were correlated to seminal BPS and AMHRII levels.

Results: qPCR revealed no differences in AMHRII, SMAD1 and SMAD4 mRNA levels between high and low BPS. AMH and AMHRII were localized to the sperm head and midpiece and their levels were reduced in high BPS groups ($p<0.05$ and $p<0.01$, respectively). No significant relationship was observed between seminal BPS and all SMAD protein expression, which were localized to the sperm head only.

Seminal BPS was negatively correlated with sperm count (r=0.2728; p=0.029), but not with motility, morphology, or DNA fragmentation. AMHRII levels were positively correlated with sperm count (r=0.4837; p<0.001), motility (r=0.2577; p=0.022) and morphology (r=0.3263; p=0.011).

Conclusion: This is the first study to correlate AMHRII with semen parameters. Co-localization of AMH and AMHRII on the head and midpiece further supports a functional role in sperm. Future experiments aim to establish a physiological role of AMH in sperm and determine if it can mitigate BPS exposure.

Funding: NSERC, CFAS, OGS and OVC.

OP7. miR-to-miR interactions: BPA and its analogs dysregulate key miRs and developmental parameters in a miR-21 independent manner in COCs and *in vitro* produced embryos

Mcilhargey-Larkin, F.K[1]., Sabry, R.[1], Favetta, L.A.[1]

[1]Reproductive Health and Biotechnology Lab, Department of Biomedical Sciences, OVC, University of Guelph, Guelph, ON, Canada

Despite improvements in artificial reproductive technologies (ARTs), fertility is declining with a contributing factor being exposure to endocrine disrupting compounds (EDCs). Bisphenol A (BPA) and analogs, BPS and BPF, are EDCs associated with reproductive dysfunction. BPA significantly increases miR-21 in cumulus oocyte complexes (COCs). microRNAs (miRs) are short noncoding epigenetic regulators influencing developmental processes. miR-to-miR interactions provide an additional layer of gene regulation. miRs are compensated by other miRs to preserve regulatory functions and cooperatively control shared targets. Three key steps for successful *in vitro* embryo production are oocyte maturation, embryonic genome activation (EGA) and blastulation. We assessed effects of miR-21 downregulation and bisphenols' exposure on miRs in COCs, arrested 8-cell embryos and blastocysts, as we hypothesise that BPA's analogs affect miRs key for development in a miR-21-dependent manner. Bovine COCs were matured for 24 hours with/without anti-miR-21 and with/without BPA, BPS or BPF at the LOAEL dose of 0.05mg/ml. COCs were snap-frozen in liquid nitrogen for downstream analysis or *in vitro* fertilized. At day 8 post-insemination blastocysts and arrested 8-cell embryos were collected and crucial miRs (miR-10b, -21, -29a, -34c, -103a, -130a, -155, and -378) were quantified by qPCR. COCs maturation rate was determined by polar body extrusion: all groups treated with anti-miR21 and BPA, BPS or BPF displayed a significant decrease in maturation (p<0.0001). Cleavage rates were reduced by anti-miR-21 (p=0.02), BPA alone/anti-miR-21 BPA (p=0.0006, 0.002), and BPF alone/with anti-miR-21 (p=0.02, 0.04). Blastocyst rates declined in anti-miR-21 (p=0.004), anti-miR-21 with BPA, BPS and BPF (p=0.0003, 0.06, 0.01) and BPA alone (p=0.001). DNA fragmentation was assessed by TUNEL to determine blastocyst quality with anti-miR-21 and bisphenol groups displaying increased fragmentation (p=0.01-5.4x10^{-5}). These results highlight EDCs' effects on miR-to-miR interactions and *in vitro* pre-implantation development in a miR-21 independent manner, ultimately affecting ARTs outcome. Funding Sources: NSERC, OGS, OVC.

OP8. Protein Dysregulation in Pre-eclampsia: Precision Peptide-Based Molecular Insights

Aditi Kini[1], Janice Corbette[2], Faith Whitehead[1], Yaseen Ahmed[1], Moustapha Alayan[1], Ashkan Golshani[2], Maha Othman[1,3,4], **Thomas DD Kazmirchuk[1]**

[1]Department of Biomedical and Molecular Sciences, Queen's University; Kingston, Ontario, K7L 3N6, Canada, [2]Department of Biology, Carleton University; Ottawa, Ontario, K1S 5B6, Canada, [3]School of Baccalaureate Nursing, St. Lawrence College; Kingston, Ontario, K7L 5A6, Canada, [4]Clinical Pathology Department, Faculty of Medicine, Mansoura University; Mansoura, 35516, Egypt.

Background: Pre-eclampsia (PE) is a pregnancy-specific placental disorder affecting 2–8% of pregnancies globally, remaining a major cause of maternal and perinatal mortality, causing approximately 70,000 maternal and 500,000 neonatal deaths annually. Defined by new-onset hypertension and end-organ complications, PE involves placental and endothelial dysfunction. Its molecular mechanisms remain enigmatic. Dysregulation of circulating and vascular proteins is increasingly recognized as driving pathogenesis. This study aims to systematically identify proteins involved in PE to better understand its pathophysiology and inform future diagnostics.

Methods: This systematic review follows PRISMA guidelines. Literature searches using PubMed, Embase, and Web of Science are underway. Independent reviewers screened studies in two stages (title/abstract and full-text). Eligible studies report protein biomarkers or mechanistic protein alterations in human PE; non-human studies, reviews, and studies lacking extractable protein data were excluded. Proteins were categorized by biological system with emphasis on reproducibility across studies. Screening and data extraction are ongoing.

Results: Preliminary data indicates proteins across placental (PIGF, VEGF, sFLT-1), metabolic (Ghrelin, GHSR-1), circulatory (Protein C, Protein C receptor), and vascular/hemostatic (VWF, ADAMTS13) systems were consistently implicated in PE. Dysregulation of angiogenic and endothelial pathways is recurrent. Several studies report increased VWF and reduced ADAMTS13 activity, indicating vascular homeostasis and coagulation perturbations.

Conclusion: A possible systemic protein dysregulation characterizes PE. This supports endothelial and hemostatic imbalance as central to disease pathophysiology, highlighting protein-based biomarkers for prediction and stratification. VWF–ADAMTS13 axis disruption may contribute to broader vascular dysfunction in PE. Further pathway evaluation may guide the development of approaches for risk and disease-severity assessment or early detection.

Funding: None

11:45-12:00 FLASH TALKS

FT1. Accelerating Aptamer Discovery for Bovine Sperm Sexing using a Data-driven Non-Iterative SELEX Framework

Nikita Gahoi[1,3], Soham Sassan[1,3], Veronika Magdanz[1] and Runjhun Narayan[1,2,3,4]

[1]University of Waterloo, Canada, [2]The University of British Columbia, Canada, [3]MOLwise Biosciences Inc., [4]ICASSSD*, Canada Corresponding author: runjhun.saran@uwaterloo.ca

Background and objectives: Efficient separation of X- and Y-chromosome-bearing spermatozoa is critical for sex-specific livestock breeding. Current flow cytometry-based methods rely on fluorescent DNA staining (e.g., Hoechst dyes) in spermatozoa, making them costly, technically demanding, potentially harmful to sperm viability, and prone to genetic abnormalities in offspring. DNA aptamers, short single-stranded oligonucleotides, offer a label-free and non-invasive alternative due to their high specificity for cellular targets. Conventional SELEX approaches are labor-intensive, time-consuming, and prone to bias. Here, we present a data-driven, non-iterative one-round SELEX framework combined with computational analysis to identify DNA aptamers that selectively bind X- or Y- bovine spermatozoa.

Methods: The N30 ssDNA library (900 pmol; ~12–15 copies per sequence) was split into two and incubated with 10^5 sorted X- or Y-spermatozoa (>75% viability) respectively in Tris-based buffer at RT for 45 min. Spermatozoa were then passed through a 0.2 µm filter to separate unbound DNA from spermatozoa retained on the membrane. Eight sequential washes removed weak and non-specific binders. Strongly sperm-bound sequences were recovered via EDTA elution, followed by cell lysis to obtain tightly associated DNA. DNA from all fractions was amplified and sequenced (NGS). Differential Sequence enrichment and k-mer frequencies were normalised and assessed using fold-change and

statistical testing. Logistic Regression and Random Forest models were applied to classify and rank discriminative features, which were mapped back to full sequences to identify cell-type-specific motifs.

Results: DNA across all washes and elution fractions was recovered and sequenced (~32,000 reads per fraction). Ongoing computational analysis indicates distinct sequence patterns between X- and Y-sperm DNA populations. Differential abundance profiling is enabling identification of candidate aptamers with selective binding signatures.

Conclusion: This study establishes a significant step towards a scalable, fertility-preserving alternative to conventional sperm sorting, while accelerating aptamer discovery via a fast, reliable, time and cost-effective SELEX strategy.

Funding: MITACS Accelerate Grant

FT2. Triphenyl phosphate disrupts estrogenic, glycolytic and lipid homeostasis: integrated meta-analysis across five vertebrate species and functional validation in aquatic embryonic cells

Logan Germain, **Holly Mackay**, Taylor Bird, Lihua Xue & Louise M Winn

Background: Triphenyl phosphate (TPhP) is a popular flame retardant and plasticizer that is pervasive in the environment, resulting in frequent human and ecological exposure. There is growing epidemiological and experimental evidence linking exposure to metabolic and endocrine dysfunction, including obesity, diabetes and even certain cancers. However, its molecular mode of action remains uncertain and fragmented across different species and studies. Fish models are valuable alternative systems in developmental toxicology due to their environmental relevance, conserved signaling pathways, dynamic epigenome, and ethical advantages.

Methods: We integrated five transcriptomic datasets profiling TPhP exposure in human ovarian granulosa cells (KGN), primary mouse Leydig cells, medaka embryos, zebrafish embryos, and a rainbow trout embryonic cell line (STE-137). Differential expression analysis was performed, genes were converted to human orthologs then combined using a weighted Stouffer meta-analysis approach. Conserved genes ("the gold set") were defined by meta-FDR < 0.05, detection in ≥ 3 studies, and directionally concordant. The gold set genes were analyzed *in silico* for protein network interactions and functional enrichment analysis in STRING-DB. Follow-up functional validation studies of *in silico* findings were performed in STE-137 cells using 17β-estradiol ELISA, L-lactate ELISA and LipidTOX staining via flow cytometry at 24, 48, and 72 h of TPHP exposure.

Results: We identified 618 conserved TPhP-responsive genes across 5 vertebrate species. Protein network and functional enrichment analysis of these genes revealed conserved alterations to translation machinery, estrogen metabolism, sterol biosynthesis, calcium signaling and mitochondrial matrixes. Functional validation in STE-137 cells confirmed: 17β-estradiol production was significantly elevated at 48h of exposure ($p < 0.01$), L-lactate accumulation was increased at 48h and sustained at 72h ($p < 0.05$), consistent with upregulation of glycolytic enzymes (PKM, ENO2) in the gold set gene list, and lipid storage was nearly doubled at 24h of TPHP exposure ($p < 0.01$).

Conclusions: TPhP exposure causes a conserved transcriptional response across 5 vertebrate species and study models. These conserved responses converge on endocrine and metabolic function, with functional validation confirming these findings in an aquatic embryonic cell model. These alterations during embryonic development highlights a potential mechanism by which environmental exposures may influence long-term disease susceptibility.

FT3. Platelet GPIbα Dysfunction Is Associated with Abnormal Placental Structure and Adverse Pregnancy Outcomes

Vrisha Shah[1], Harmanpreet Kaur[1], Maha Othman[1,2,3]

[1]Department of Biomedical and Molecular Sciences, School of Medicine, Queen's University, Kingston, Ontario, Canada; [2]School of Baccalaureate Nursing, St Lawrence College, Kingston, Ontario, Canada; [3]Clinical Pathology Department, Faculty of Medicine, Mansura University, Egypt

Platelet function disorders are associated to increased risk of pregnancy complications. Platelets are crucial in placentation, vascular development, and tissue remodeling, ensuring adequate nutrients delivery to fetus. Disruption of these processes may contribute to adverse pregnancy outcomes. Platelet-type von Willebrand disease (PT-VWD), caused by G233V mutation in glycoprotein Ib alpha (GPIbα) receptor, results in hyperresponsive platelet binding to von Willebrand factor without hemostatic trigger of shear stress. This leads to premature clearance of platelet–VWF complexes and reduced platelet availability at vascular remodeling sites, suggesting a mechanistic link between platelet dysfunction and abnormal placentation. We evaluated placental development and fetal growth in transgenic mouse models with altered GPIbα function.

Three maternal transgenic strains lacking native GPIbα were used: humanized wildtype (hTg-WT; hGPIb+/+), humanized mutant (hTg-MT; hGPIbG233V+/+; PT-VWD model) and null (NULL; hGPIb−/−). Dams were sacrificed at embryonic days (E)12.5 and E18.5. Fetal and placental weights were recorded, and placental structure was assessed using H&E staining and microscopy.

At E12.5, MT fetuses weighed less than WT and NULL and resolved by E18.5, suggesting compensatory adaptation. Placental weights were similar at E12.5, indicating structural differences. Histology showed WT placentas had mature vasculature, with capillaries and organized blood spaces. In contrast, MT and NULL placentas showed dense trophoblast regions, disorganized blood spaces, and immature vasculature.

Findings indicate early placental inefficiency in MT mice that normalizes later, supporting a role for GPIbα in early placental vascular development and highlighting potential pathways, including VEGF-mediated mechanisms.

This project received funding from the Canadian Hemophilia Society and the Arts & Science Undergraduate Research Fund at Queen's University.

FT4. Enhancing Early Obstetric Ultrasound Competency in Medical Education: A Resident-Led POCUS Workshop Evaluation

Natalie Kearn*[1], Hannah Brown*[1], Danielle Campagnolo[2], **Fariya Zaheer[1]**, Annalise Gignac[1], Nooh Kabir[1], Stanley Chen[1], Aurelia Zhou[1], Joseph Newbigging[1]
*these authors contributed equally

[1]Department of Emergency Medicine, Faculty of Health Sciences, Queen's University, Kingston, Ontario, Canada, [2]Department of Obstetrics & Gynecology Faculty of Health Sciences, Queen's University, Kingston, Ontario, Canada

Background: Early pregnancy assessment and fetal well-being evaluation are central to reproductive and obstetric care. Point-of-care ultrasound (POCUS) is increasingly utilized in these settings; however, undergraduate medical exposure to obstetric ultrasound remains limited. This study evaluates whether a resident-led, student-organized obstetric POCUS workshop improves pre-clerkship medical students' confidence and competency in core reproductive imaging skills.

Methods: First- and second-year medical students participated in a 90-minute workshop delivered in small groups by OB/GYN residents. Sessions included a didactic introduction followed by supervised hands-on practice with pregnant volunteers. Core competencies included identification of fetal cardiac activity (m-mode), fetal lie, placental location, and amniotic fluid assessment. Pre-, post-, and 3-month follow-up surveys assessed confidence, knowledge, and skill retention. Quantitative data were analyzed using paired statistical tests, while qualitative feedback underwent thematic analysis.

Results: Seventy-two students enrolled (38% first-year, 63% second-year). Baseline ultrasound exposure was limited, with most participants reporting only 1–2 prior sessions. Preliminary findings suggest significant improvements in self-reported confidence and ability to identify key obstetric structures immediately post-workshop, with sustained competency trends anticipated at follow-up. Participants highlighted the value of hands-on exposure and near-peer teaching in understanding early pregnancy imaging.

Conclusion: A structured, resident-led obstetric POCUS workshop enhances early learner confidence and foundational skills in reproductive ultrasound. Integrating such training into pre-clerkship curricula may strengthen competency in early pregnancy assessment and improve readiness for clinical obstetric care.

Funding: No external funding was received.

12:00 – 1:00: Lunch

1:00 – 1:30: Keynote Presentation:

Dr. Graeme Smith, Queen's University
"*Pregnancy Complications and Future Cardiovascular Disease*"

1:30 – 3:00: Poster Session – SOM Atrium

- Even numbers 1:30 – 2:15
- Odd numbers 2:15 – 3:00

3:00 – 3:45: Oral Presentations III – *Chair: Dr. Steve Renaud*

OP9. Cyclosporin A treatment rescues mitochondrial apoptotic marker expression in a hypoxia-induced syncytiotrophoblast stress model of preeclampsia

Jenny S. Feeney[1], Tina Podinic[1], Cristina Monaco[1], Yiye Wang[1], Samantha L. Wilson[2], Sandeep Raha[1]

[1]Department of Pediatrics and the Graduate Program in Medical Sciences, McMaster University, Hamilton, Ontario, Canada, [2]Department of Obstetrics and Gynecology, McMaster University, Hamilton, Ontario, Canada

Background/objectives: Preeclampsia (PE) is a leading cause of maternal and fetal mortality, with limited treatment options. Placental hypoxia is implicated in PE pathogenesis, driving anti-angiogenic factor release, mitochondrial dysfunction, and intrinsic (mitochondrial) apoptosis. Cyclosporin A (CsA) has been shown to modulate mitochondrial function and apoptotic signalling pathways. We hypothesized that hypoxic exposure of blastocyst-derived syncytiotrophoblasts (STs) would model ST stress observed in PE, and that CsA would mitigate this stress by limiting mitochondrial damage. We aimed to (1) establish hypoxic conditions that reliably induce PE-like ST stress and (2) evaluate the efficacy and mechanisms of CsA in attenuating this response.

Methods: RCB 4940 blastocyst-derived human stem cells were differentiated into STs and pre-treated with 1 μM CsA. Following differentiation, cells were exposed to physiological (8%) or hypoxic (1%) O_2 conditions for 24 hours. Apoptosis and PE-associated angiogenic markers were assessed using Annexin V/propidium iodide (PI) fluorescence microscopy, RT-qPCR, and Western blotting.

Results: Exposure to 1% O_2 induced apoptotic signalling – evidenced by increased Annexin V/PI staining and expression of intrinsic apoptotic markers *BAX*, *Caspase 9*, and p53 – and PE-like angiogenic marker expression, as seen through increased *sFlt-1* and *sEng* expression, relative to 8% O_2 controls. CsA treatment attenuated these observed apoptotic and PE-like biomarker changes.

Conclusion: CsA mitigates hypoxia-induced ST stress, likely through modulation of mitochondrial apoptotic pathways. These findings implicate mitochondrial apoptotic signalling in PE-like ST dysfunction and support mitochondrial-targeted therapies as a potential treatment strategy.

Funding: CIHR (CGS-M Scholarship), NSERC, New Frontiers Research Grant

OP10. OVOL2 Reinforces Epithelial Identity and Mediates Syncytiotrophoblast Formation in the Mouse Placenta

Violet S. Patterson[1], Mariyan J. Jeyarajah[1], Stephen J. Renaud[1, 2, 3, *]

[1]Department of Anatomy and Cell Biology, University of Western Ontario, London, [2]Children's Health Research Institute, [3]Lawson Health Research Institute, * Experiments in this study were supported by the Natural Sciences and Engineering Research Council of Canada (NSERC), with additional support from the Canadian Institutes of Health Research.

Background: The placenta is comprised of trophoblast cells, and failure to maintain their epithelial identity can impair placental development. In mice, distinct trophoblast lineages populate two anatomically and functionally distinct regions: the junctional zone and labyrinth zone (LZ). The transcriptional repressor OVO-like 2 (OVOL2), a known regulator of epithelial identity, is predominantly expressed in LZ syncytiotrophoblast (SynT) cells. Embryos lacking OVOL2 exhibit impaired placental development, leading to embryonic lethality. We therefore hypothesize that OVOL2 regulates SynT lineage formation by preserving epithelial identity during trophoblast stem (TS) cell differentiation.

Methods: Pregnant mice were sacrificed at embryonic day (E)9.5 and E12.5. Placental tissue was collected for immunofluorescence to localize trophoblast lineages and epithelial/mesenchymal markers. Wild-type and *Ovol2*$^{-/-}$ TS cells were differentiated toward LZ lineages using CHIR99021. Gene expression and pathway enrichment were assessed by bulk RNA sequencing. SynT formation was assessed via fusion index, and genomic OVOL2 binding was evaluated by chromatin immunoprecipitation. $P<0.05$ was considered statistically significant.

Results: When cultured in LZ differentiation conditions, both wild-type and *Ovol2*$^{-/-}$ TS cells exhibited reduced stem marker expression (*Cdx2*, *Eomes*, and *Esrrb*; $P<0.05$); however only wild-type cells showed increased expression of SynT markers (*Synb*, *Gcm1*, and *Syna*; $P<0.05$), increased fusion ($P<0.05$), and enrichment of gene ontology terms such as "placenta development" (NES=1.68). OVOL2 bound proximal to genes associated with mesenchymal identity (*Id1, Zeb1* and *Vim*). Top upregulated gene ontologies in differentiating wild-type trophoblasts were associated with epithelial morphogenesis, whereas those upregulated in *Ovol2*$^{-/-}$ cells included upregulated mesenchyme development ($Q<0.01$). *Ovol2*$^{-/-}$ placentas showed reduced E-Cadherin, MCT1, and MCT4 compared to placentas with *Ovol2* and conversely show stronger Vimentin staining; however, E-Cadherin and Vimentin were identified in cells proximal to MCT4-positive staining in E12.5 placentas.

Significance: These findings indicate that OVOL2 is a key regulator of SynT lineage formation and LZ development by safeguarding epithelial identity.

OP11. Fluoxetine-Induced Oxidative Stress Disrupts Tryptophan and VEGFA Signaling in Human Placenta Cells

Rodrigo Vargas[1], Abby Delamare[1], Alison C. Holloway[1]

[1]Department of Gynecology and Obstetrics, McMaster University, Hamilton, ON, Canada

Introduction: Major Depressive Disorder (MDD) is a prevalent global health concern, aAecting over 10% of women during the perinatal period. Untreated MDD is associated with significant obstetric complications, including placental dysfunction, preeclampsia, preterm birth (PTB), and low birth weight (LBW). Selective Serotonin Reuptake Inhibitor (SSRI) antidepressants, specifically fluoxetine (Prozac®), are the most prescribed pharmacological treatment for MDD in the perinatal period. However, concerns persist regarding their impact on neonatal outcomes as SSRI use during pregnancy has been associated with adverse pregnancy outcomes including PTB and LBW. Although PTB and LBW have been linked to deficits in early placentation and/or placental function, the eAects of SSRIs on the placenta are largely unknown. This study aims to elucidate the mechanisms by which fluoxetine exposure can impact placental trophoblast cell function.

Methods: First trimester human placental trophoblast cells (HTR-8/SVneo) were treated with fluoxetine hydrochloride across a broad range of physiological concentrations (0.1, 0.5, 1, 5, and 10uM) for 48hs. We assessed cytotoxicity, markers of oxidative stress, DNA repair, angiogenesis and alterations in tryptophan metabolism.

Results: Fluoxetine treatment increased the production of reactive oxygen species in association with decreased expression of angiogenic markers. There were profound changes in tryptophan metabolism and cell cycle regulatory genes.

Conclusion: In the placenta, oxidative stress is fundamentally linked to reduced perfusion and compromised vascular permeability, which collectively impair the delivery of essential nutrients and oxygen to the fetus. Alterations in tryptophan homeostasis have been associated with adverse pregnancy outcomes and sub-optimal fetal neurodevelopment. While the present study demonstrates a correlation between fluoxetine exposure and redox imbalance, attenuated vascularization, and altered tryptophan metabolism, further research is required to comprehensively evaluate the long-term impact of antidepressant pharmacotherapy on perinatal outcomes.

3:45 – 4:15: Break - Refreshments

4:15 – END: AWARDS and Farewell

Poster Abstracts

P1. Early Global Suppression and Later Functional Reprogramming of the Placenta Following Benzene Exposure

Megan E. Cull[1], Julia Rioux[1], Lauren Brown[1], Lihua Xue[1], Louise M. Winn[1,2]

[1]Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, [2]School of Environmental Sciences, Queen's University, Kingston, Ontario.

Background: Benzene is a widespread environmental pollutant associated with cancer and adverse developmental outcomes; however, its early effects on placental function remain poorly understood. This study investigated the acute and sustained effects of benzene exposure on placental gene expression across key functional domains.

Methods: Pregnant CD-1 mice were exposed to corn oil (control) or 200 mg/kg benzene from gestational days 8–14, and placentas were collected at 2 hours, 24 hours, and 5 days post final exposure. Gene expression was assessed using qPCR targeting markers of trophoblast differentiation, proliferation, and nutrient transport.

Results: At 2 hours post-exposure, benzene induced a broad increase in Cq values across all gene categories, consistent with a rapid and global suppression of structural, progenitor, proliferative, and nutrient transporter-associated gene expression. By 24 hours, this response became more selective, with persistent downregulation of labyrinth structural trophoblast markers, including *Syna* and *Ctsq*, indicating sustained impairment in differentiated trophoblast populations. In contrast, increased expression of the labyrinth-associated progenitor marker *Epcam* and folate transporter *Slc19a1* was observed. At 5 days post-exposure, benzene-exposed placentas exhibited continued disruption of trophoblast differentiation, with reduced expression of key structural markers (*Syna* and *Prl8a8*), and altered proliferative dynamics, characterized by decreased *Mki67* and increased *Pcna* expression. In parallel, nutrient transport pathways showed marked reprogramming, including reduced folate transporter expression alongside increased expression of lipid and amino acid transporters. Notably, sex-specific differences emerged over time, particularly within transporter-associated genes.

Conclusions: These findings demonstrate that benzene exposure elicits a rapid global suppression of placental gene expression followed by selective recovery and sustained functional reprogramming. This disruption of placental differentiation, proliferation balance, and nutrient transport capacity may contribute to altered fetal development following environmental toxicant exposure.

Funding: OGS and CIHR

P2. The Impact of Oxygen Tension on Human Placental Pericyte Fidelity

Emily Suggitt, Bryony V. Natale, David R.C. Natale

Department of Biomedical and Molecular Sciences, Queen's University

Background: Pericytes play a crucial role in vessel integrity and sprouting angiogenesis within the placenta. They also display plasticity, but this is context dependent; based on the cellular exposures in the placenta, placental pericyte fate is dedifferentiation to mesenchymal stem cells (MSC), differentiation to fibroblasts, or phenotypic switching to vascular smooth muscle cells (vSMC). Preservation of pericyte phenotype and function indicates maintained pericyte fidelity. KLF4 has previously been shown as a potential supporting marker to recognize changes in fidelity. Abnormal variations in oxygen tension could be a stressor on the placental environment and challenge fidelity. It is unknown whether oxygen alone may be sufficient to disrupt fidelity of pericytes in the placenta.

Methods: Transcriptional analysis was completed on cultured human placental pericyte populations from four different oxygen thaw/culture parameters: 20/20%, 20/8%, 8/8%, and 8/2% O_2.
qPCR assessed the presence of pericyte, fibroblast, MSC, and vSMC markers, along with the potential supporting fidelity marker KLF4. Subsequent protein analysis of PDGFRb, NG2, NES, and aSMA was used to evaluate morphological and cell-specific presence of pericyte markers.

Results: vSMC marker *MYH11* was absent at each oxygen parameter. Transcriptional expression of MSC and fibroblast markers was correlated to the presence of pericyte markers at the transcript and protein level. Qualitatively, 99% of cells expressed 2 pericyte markers, PDGFRb and aSMA while 95% expressed NG2 and NES in our immunocytochemical assessments.

Conclusion: Pericyte fidelity is maintained at 20/20%, 20/8%, 8/8%, and 8/2% oxygen thaw/culture, demonstrating that placental pericyte fidelity remains robust when the only environmental trigger is O_2. *KLF4* reflected preservation of fidelity with consistent expression across each oxygen level.

Funding: Canadian Institutes of Health Research

P3. Deep Learning Semantic Segmentation of Fetal and Maternal Blood Spaces in Rat Placental Histology

Ruslan Amruddin[1], Naween Sarwari[2], Savvy Liu[3], Bryony V. Natale[1], David R.C. Natale[1]

[1]Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada [2]Faculty of Computer Science, Western University, London, ON, Canada [3]Faculty of Computer Science, McMaster University, Hamilton, ON, Canada

Background and Objectives: Quantitative analysis of placental vascular architecture is limited by subjective, lengthy manual annotation, while existing automated tools measure staining intensity rather than architecture and cannot separate structurally distinct compartments with similar staining. We developed and validated a deep learning pipeline for semantic segmentation of fetal blood spaces (FBS), maternal blood spaces (MBS), and background tissue in CD31-stained rat placental histology at embryonic day 19.5.

Methods: Each pipeline component was optimized through sequential experiments that compared encoders (including histology-pretrained vision transformer foundation models such as Phikon-v2, UNI, CONCH, and Virchow2), decoders, loss functions, and augmentation strategies. Data efficiency was assessed across increasing training set sizes. Downstream morphometric validation compared model and expert measurements on 25 held-out images from 5 placentas, with splits enforced at the placenta level, across blood space area fractions, FBS:MBS area ratios, and FBS perimeter-to-area ratios.

Results: ImageNet-pretrained ConvNeXt-Small outperformed all histology foundation models at an order of magnitude lower inference cost. Loss function and augmentation choices had minimal impact (Dice spread 0.008). The final pipeline achieved mean Dice 0.821 at 14.9 ms per tile, plateauing at 5 to 7 placentas (97% of full-dataset accuracy). Morphometric measurements showed strong agreement with expert annotations: ICC 0.90 for blood space area fractions, 0.80 for FBS:MBS ratio, and 0.56 for FBS perimeter-to-area ratio, with no significant systematic bias on any metric.

Conclusion: Standard ImageNet-pretrained encoders, minimal augmentation, and roughly 25 annotated images suffice for accurate, unbiased quantitative morphometry of placental vasculature. By replacing 30 to 60 minutes of manual tracing per image with near-instant inference (14.9 ms per tile), the pipeline makes high-throughput placental analysis practical for toxicological and pregnancy-related disease research.

Funding: CIHR

P4. PFOA Exposure Reduces Polar Body Extrusion During In Vitro Oocyte Maturation

Mohr, SA., Galvao, NA., Favetta, LA.

Reproductive Health and Biotechnology Lab, Department of Biomedical Science, OVC, University of Guelph, Guelph, ON.

Background: Emerging research has linked per- and poly-fluoroalkyl substances (PFAS) exposure to altered gamete viability and infertility. PFAS are a class of industrial chemicals, classified as endocrine-disrupting compounds (EDCs), with agonist characteristics of endogenous hormones through receptor mediated disruption. Due to their toxic effects, the Government of Canada has limited PFAS use since 2007. However, these chemicals are still prevalent in the environment due to their long half-life (forever chemicals). Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) are the most

abundant members of the PFAS family, with few studies available especially on the role of PFOA on fertility and early development. Here we hypothesized that PFOA at environmentally and physiologically significant doses affects oocyte maturation, measured by polar body extrusion (PBE).

Methods: Cumulus-oocyte complexes (COCs) were aspirated from bovine ovaries obtained from a local slaughterhouse and allocated to one of four groups; control, 0.01mg/mL PFOA, 0.05mg/mL PFOA, or 0.075mg/mL PFOA. COCs were matured for 24-hours, denuded and oocytes were fixed using 1% PFA. PBE was assessed under microscopy.

Results: A significant decrease in PBE rate was observed among oocytes in the 0.01mg/mL (p=0.008), 0.05mg/mL (p=0.024) and 0.075mg/mL (p=0.011) groups compared to the control (52.1% PBE). PBE rates of 29.0%, 34.0%, and 29.9% were observed in the 0.01mg/mL PFOA, 0.05mg/mL PFOA, and 0.075mg/mL PFOA groups, respectively.

Conclusion: To our knowledge, this is the first study to show the effects of PFOA exposure during in vitro bovine oocyte maturation. Bovine oocytes were used as translational model to human. These very preliminary results suggest that PFOA interferes with oocyte maturation, via a nonmonotonic dosing mechanism. Future experiments will investigate spindle quality and formation, as well as its effects on cleavage rates and blastocyst development to further assess the role of these forever chemicals on fertility and embryo development.

P5. (FT1) Accelerating Aptamer Discovery for Bovine Sperm Sexing using a Data-driven Non-Iterative SELEX Framework

Nikita Gahoi[1,3], Soham Sassan[1,3], Veronika Magdanz[1] and Runjhun Narayan[1,2,3,4]

[1]University of Waterloo, Canada, [2]The University of British Columbia, Canada, [3]MOLwise Biosciences Inc., [4]ICASSSD*, Canada

**Abstract included with flash talk abstracts*

P6. Inflammation-induced fetal loss is associated with reprogramming of maternal bone marrow monocytes and fetal growth restriction in subsequent pregnancies

Nakeisha A. Lodge-Tulloch, Charles H. Graham, and Tiziana Cotechini.

Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada.

BACKGROUND: Pregnancy complications, such as fetal loss, are associated with aberrant maternal inflammation, increased release of damage-associated molecular pattern (DAMP), and an elevated risk of subsequent pregnancy complications. We hypothesize that aberrant inflammation during pregnancy causes pathological reprogramming of maternal innate immune cells leading to subsequent pregnancy complications.

OBJECTIVE: To determine whether aberrant maternal inflammation associated with fetal loss alters the reprogramming of maternal bone marrow monocytes and increases the risk of complications in subsequent pregnancies.

METHODS: To induce aberrant inflammation in pregnancy, C57BL/6 mice were administered 20 µg/kg of lipopolysaccharide (LPS) or saline intraperitoneally on gestational day (GD) 10.5. Dams were euthanized on postnatal day (PD) 7 or bred for a second pregnancy. Litter size and pup weight were recorded on PD 0 and 7. Whole bone marrow and isolated bone marrow monocytes harvested on PD 7 were challenged *in-vitro* with LPS or Pam_3Cys. Cytokine concentrations in cell-free supernatants were measured using a multiplex platform.

RESULTS: Maternal exposure to LPS reduced litter size and pup weight. Pups born to dams exposed to LPS in the preceding pregnancy were significantly smaller. Functionally, whole bone marrow from

dams previously exposed to LPS showed altered cytokine production following secondary stimulation, including reduced TNF and GM-CSF levels. Similarly, bone marrow monocytes from these dams exhibited increased IL-6 and decreased IL-1β production.

CONCLUSION: Aberrant inflammation during pregnancy causes pathological maternal immune reprogramming that may increase risk of complications in subsequent pregnancies. Further understanding of the mechanism of pathological reprogramming during pregnancy may identify targets to mitigate long-term risk of complications.

P7. Characterizing Stem cell antigen-1 (Sca-1) expression in trophoblast stem cells and mammary carcinoma cells.

Abigail J. Koshan, Bryony V. Natale, Tiziana Cotechini, David R. C. Natale.

Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada.

Background: Stem cell antigen 1 (Sca-1) is a widely used marker for the enrichment of mouse stem and progenitor cell populations. We previously demonstrated that Sca-1 identifies a trophoblast population with multipotent potential in the mid-gestation mouse placenta. Sca-1 has also been associated with tumor-initiating cells and has emerged as a potential regulator of stem cell-like behaviors including multipotency and proliferative potential, in cancer. A hallmark of cancer is epithelial to mesenchymal transition (EMT), characterized by upregulation of Ncadherin and downregulation of E-cadherin. Here, we propose to characterize the temporal dynamics of Sca-1 expression in mouse trophoblast stem (mTS) cells and mammary carcinoma cells to determine whether Sca-1 expression is associated with conserved phenotypes across these cell types.

Methods: Sca-1 expression in mTS, 4T1 and 66cl4 cells was examined by qPCR and flow cytometry every 24 hours over 72 hours of growth. To evaluate EMT, expression of *Cdh2* (Ncadherin)*, Cdh1* (E-cadherin), *Vim* (Vimentin), were evaluated by qPCR in 4T1 and 66cl4 cells at 24h, 48h, and 72h of growth.

Results: Sca-1 expression patterns differed between mTS, 4T1 and 66cl4 cells, where expression decreased over 72h in mTS and 66cl4, and increased in 4T1 cells. mTS cells highly express Sca1 after 24h (96.9%), 48h (98.5%), and 72h (91.6%), compared to 4T1 (13.9% at 24h, 30.4% at 48h, 32.3% at 72h) and 66cl4 cells (4.8% at 24h, 6.4% at 48h, 2.6% at 72h). Analysis of EMT marker expression showed greater relative *Cdh1* expression in 4T1 than 66cl4 cells, consistent with an epithelial phenotype and greater relative *Cdh2* expression in 66cl4 than 4T1 cells, consistent with a mesenchymal phenotype.

Conclusions: These findings suggest Sca-1 expression patterns are not conserved between trophoblast and cancer cell populations and Sca-1 is more highly expressed in mammary carcinoma cells possessing an epithelial phenotype.

Funding: NIH/NICHD

P8. Investigating the Functional and Metabolic Deficits Resulting from Δ9-Tetrahydrocannabinol Exposure in Differentiating Human Myotubes.

Christian Natale and Daniel B. Hardy

Department of Physiology and Pharmacology, Schulich School of Medicine and Dentistry, Western University.

Background: Δ9-tetrahydrocannabinol (THC) exposure during pregnancy can lead to fetal growth restriction which is suggested to hinder muscle growth and development. Moreover, ∆9-THC-exposed rat offspring exhibit overall glucose intolerance and insulin resistance within the muscle. While the endocannabinoid system has been implicated in the regulation of myogenic fusion and metabolism in skeletal muscle, little is known about the impact of Δ9-THC exposure during myogenesis on skeletal muscle development or function. Preliminary findings in L6 rat myoblasts have shown that ∆9-THC

exposure during myogenesis alters the expression of genes associated with the development of skeletal muscle, metabolic function and cellular response to oxidative stress. We **hypothesize** that during myogenesis, Δ9-THC directly impairs both myogenic fusion and metabolic function.

Methods: We will use a translatable model of ∆9-THC exposure during myogenesis, using primary human myoblasts. Cells will be exposed to a low or high physiological dose of ∆9-THC with or without selective cannabinoid receptor antagonists. Supplementation will occur through growth and differentiation, followed by a treatment free period. Cells will be assessed at the beginning and end of this period for changes in (1) myoblast fusion and myogenic protein abundance; (2) the insulin signaling pathway (Akt/ PI3K cascades); (3) oxidative metabolism and (4) glucose uptake in response to insulin.

Results: We expect ∆9-THC exposure to result in reduced myogenic protein abundance through impaired myoblast fusion and myotube maturation. Lasting reductions in insulin signaling activity and glucose uptake in response to insulin stimulus are expected in THC treated cells.

Significance
We aim to characterize the lasting impacts resulting from ∆9-THC exposure during skeletal muscle development, and to better inform our understanding of how gestational THC exposure may impact glucose and insulin homeostasis in postnatal life.

Supported by CIHR Project Grant R4228A28

P9. Maternal high-fat diet-induced obesity programs sex-dependent changes in hepatic lipogenic signalling and progressive liver disease in aging offspring.

Laura Reeves[1], Tatiane A. Ribeiro[1,3,4,6], Dawn M.E. Bowdish[3,5], and Deborah M. Sloboda[1-4,6].

[1]Department of Biochemistry and Biomedical Sciences; [2]Centre for Metabolism, Obesity, and Diabetes Research; [3]Farncombe Family Digestive Health Research Institute; [4]Departments of Obstetrics and Gynecology, and Pediatrics; [5]McMaster Immunology Research Centre; McMaster University, Ontario, Canada; [6]McMaster Institute for Research on Aging, McMaster University, Hamilton, Ontario, Canada.

INTRODUCTION: Our data show that maternal diet induced obesity programs offspring metabolic dysfunction and is linked to the development of progressive liver disease (including lobular inflammation and steatosis), with male offspring exhibiting the greatest disease burden. Disruption of hepatic lipogenic signaling pathways likely underlie this relationship but their regulation with aging is unclear. We tested whether maternal obesity drives sex dependent changes in hepatic lipogenic gene expression that contribute to liver disease in aging offspring.

METHODS: Female mice were fed a control (17% kcal from fat, CTL, n=15) or a high-fat diet (45% kcal from fat, MHF, n=12) two weeks before and throughout pregnancy and lactation. On postnatal day 7 (P7), litters were standardized to six pups (3 males and 3 females) and livers collected in a subset of offspring. Offspring were weaned onto a healthy diet (13% kcal from fat) at P21 and at P300 & P600, liver tissues were collected for analysis. RNA was isolated and gene expression was quantified using qPCR for key genes involved in lipid metabolism (*Acaca, Fasn, Chrebp, Serbp1*). Data was analyzed using factorial ANOVA to assess the effects of maternal diet, sex, and age, with post-hoc testing applied and Tukey adjustment as appropriate ($p < 0.05$).

RESULTS: *Srebp1* demonstrated significant effects of diet ($p_{diet} = 0.004$), age ($p_{age} < 0.001$) and a maternal diet × sex interaction ($p_{diet×sex} = 0.030$). *Chrebp* transcript levels were significantly lower in mHF offspring compared to CTL ($p_{diet} = 0.001$) with effects of age ($p_{age} = 0.02$) and an interaction of sex × age ($p_{sex×age} = 0.05$). While *Fasn* demonstrated age effects ($p_{age} = 0.002$) and a sex × age interaction ($p_{sex×age} = 0.001$) and *Acaca* a sex × age interaction ($p_{sex×age} = 0.008$). These effects were most pronounced in aged offspring with old (P600) male mHF offspring showing the clearest reduction of transcript levels. Females in contrast, showed little consistent change in lipogenic transcript levels at any age.

CONCLUSION: Maternal high fat diet induced obesity induces sex-specific alterations of hepatic lipogenic gene expression that persist and diverge with old age, coincident with the development of progressive male-biased liver disease. These findings support a model in with early life disruption of lipogenic signaling that may contribute to chronic liver pathology.

FUNDING: CIHR, Farncombe Institute, and Canadian Research Chairs Program.

P10. Establishing the role of Neuropilin-1 in mouse trophoblast stem (mTS) cells: Nrp-1 Inhibition and Self-renewal

Erika Merhar, Bryony Natale, David Natale

Queen's University, Department of Biomedical and Molecular Sciences

Background: Mouse trophoblast stem (mTS) cells are the resident trophoblast stem cell population that differentiates into the trophoblast subtypes that comprise the placenta. Self-renewal is the process by which stem cells proliferate to give rise to identical, undifferentiated daughter stem cells. The balance between mTS cell self-renewal and differentiation is a tightly regulated process that is required to support the developing placenta. Neuropilin-1 (Nrp-1) is a co-receptor that associates with and enhances downstream signalling responses to transforming growth factor beta (TGF-β) and vascular endothelial growth factor (VEGF), which contribute to the self-renewal of cancer stem cell populations. However, the role of Nrp-1 in mTS cell self-renewal has not been investigated. This study aimed to establish the role of Nrp-1 signalling in mTS cell self-renewal.

Methods: Isolated mTS cells were cultured at 8% oxygen (37°C, 5% CO_2) in proliferative culture media, with treatment by an Nrp-1 inhibitor (10μM) or a vehicle control. mTS cell self-renewal was evaluated via cell count, RNA analysis for markers of mTS cell stemness and differentiation, and histological analysis of colony formation, proliferation, and cell death (TUNEL).

Results: Nrp-1 inhibition did not significantly alter stem cell maintenance or proliferation, illustrating no change in self-renewal capacity. However, inhibition of Nrp-1 led to a reduction in cell number compared with the vehicle control, accompanied by increased $TUNEL^+$ cells and fragmented nuclei at 72 hours of culture, indicative of cell death.

Conclusion: These findings suggest that while Nrp-1 is not essential for mTS cell self-renewal, it may play a protective role in cell survival. Further investigation is required to elucidate the potential mechanisms underlying Nrp-1-driven cell survival.

P11. Innate immune memory related to pregnancy complications in bone marrow progenitor persists through differentiation into macrophages.

Phillip Stachera, Christina Ferazzutti, Charles H. Graham, and Tiziana Cotechini

Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada

Background: Innate immune memory describes the ability of myeloid cells to undergo epigenetic and metabolic reprogramming following exposure to pathogen- and dangerassociated molecular patterns (PAMPs and DAMPs) resulting in long-lasting altered immunological responsiveness. During inflammation-associated pregnancy complications (e.g. pre-eclampsia [PE]), the placenta releases excessive amounts of DAMPS. We previously demonstrated evidence of innate immune memory in peripheral blood circulating monocytes collected from women with PE compared with uncomplicated pregnancies. However, it is not known whether this memory is retained during differentiation to macrophages. We hypothesize that pregnancy complication-induced innate immune memory in monocytes is long-lasting and persists through differentiation into macrophages.

Methods: To model pregnancy complications, BALB/c female mice mated to C57Bl/6 male mice underwent a reduced uterine profusion pressure (RUPP) procedure on gestational day (GD) 14.5.

Controls received sham surgery. Bone marrow was isolated on GD 18.5 and postnatal day (PD) 7 and was differentiated into bone marrow-derived macrophages (BMDMs). To investigate functional and metabolic reprogramming, BMDMs were stimulated with Pam3Cys (10 µg/mL) and LPS (10 ng/mL) for 24 hours. Cell culture supernatant was collected, and cytokine concentrations were evaluated using a multiplex bead assay (Eve Technologies). Bulk RNA sequencing was performed on BMDMs (Plasmidsaurus).

Results: BMDMs generated from RUPP dams on GD 18.5 demonstrated significantly increased IL-10 release in response to LPS and increased expression of genes involved in oxidative phosphorylation and lipid metabolism pathways in response to Pam3Cys compared with BMDMs generated from sham controls. Moreover, BMDMs generated from RUPP dams on PD 7 demonstrated significantly increased IL-10 release in response to Pam3Cys compared with BMDMs generated from sham controls.

Conclusions: Pregnancy complications induce functional and metabolic reprogramming in bone marrow progenitor cells, which persists through differentiation into macrophages.

Funding: Cancer Research Society and CIHR

P12. Localized Drug Delivery for Uterine Cancer Therapy Using Magnetized Alginate Beads

Motahareh Shabani Dargah [1], Veronika Magdanz [1]

[1]Department of Systems Design Engineering, University of Waterloo, Waterloo, Canada.

Background and objectives: Conventional cancer therapies for uterine malignancies are often limited by non-specific drug distribution and systemic toxicity. This study aims to develop a magnetically guided, localized drug delivery system using doxorubicin(DOX)-loaded alginate beads for targeted treatment within the female reproductive tract.

Methods: Alginate beads embedded with iron oxide nanoparticles were synthesized to enable magnetic responsiveness. Next, the effects of nanoparticle concentration and bead size on drug loading efficiency were evaluated. Then, drug loading capacity was quantified, and in-vitro release studies were conducted over 10 days. Also, swelling behavior of magnetic and nonmagnetic beads was compared. Following in-vitro DOX release, the cytotoxicity effect of continuous release of DOX from magnetic alginate beads was assessed using uterine sarcoma cells. Ultimately, magnetic actuation of magnetic beads was tested in a test tube and a uterus phantom under oviduct-like viscosity conditions.

Results: Beads with a diameter of 1 mm containing 30 mg/mL iron oxide achieved a drug loading of approximately 13 µg of doxorubicin within 120 hours. Sustained drug release was observed over 10 days, with concentrations comparable to clinically relevant levels. Magnetic beads exhibited enhanced and prolonged swelling compared to non-magnetic controls. Cytotoxicity assays demonstrated effective killing of uterine sarcoma cells, with significant effects observed as early as day 4. Magnetic actuation experiments confirmed controllable movement in both test tube and uterus phantom environments.

Conclusion: Magnetized alginate beads provide a promising platform for targeted, controlled delivery of doxorubicin in uterine cancer treatment, offering potential to improve therapeutic efficacy while minimizing systemic side effects.

Funding: This work was supported by the NSERC Discovery Grant and the CBB Seed Fund.

P13. Establishing Managed and Unmanaged Oxidative Stress in Human Placental Pericytes Using Hydrogen Peroxide

Anthony A.A. D'Alessio[1], Bryony V. Natale[1], David R.C. Natale[1]
[1]Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario

Background: Placental oxidative stress results from an imbalance between reactive oxygen species (ROS) and antioxidant defences and plays a dual role in placental development. While moderate oxidative stress supports early placental vascularization and signalling, excessive stress contributes to placental dysfunction and pregnancy complications such as preeclampsia and fetal growth restriction. Pericytes are critical for maintaining placental fetal vascular integrity, yet oxidative stress in placental pericytes has not been well characterized. The objective of this study was to establish a model of managed and unmanaged oxidative stress in human placental pericytes.

Methods: Primary human placental pericytes were cultured at 8% oxygen and treated with 0– 250 µM hydrogen peroxide (H_2O_2) for 48 hours to induce oxidative stress. RNA was isolated and qPCR was performed for markers associated with oxidative stress damage and cellular response pathways.

Results: Minimal oxidative stress responses were observed between 0–10 µM H_2O_2, while moderate responses at 20–100 µM indicated cells were experiencing but managing oxidative stress. At 250 µM, significant increases in CHOP (*DDIT3*) alongside reduced protective signalling (*HSPA1A*), and elevated antioxidant response genes (*NQO1, HMOX1, GPX1*) indicated overwhelmed defence mechanisms and activation of apoptosis-associated pathways.

Conclusions: These findings establish 100 µM as a level of managed oxidative stress and 250 µM as a level of unmanaged oxidative stress in placental pericytes. This model provides a foundation for future studies assessing antioxidant rescue, including cannabinoids, to determine mechanisms underlying altered pericyte abundance in THC-exposed placentas.

P14. (FT2) Triphenyl phosphate disrupts estrogenic, glycolytic and lipid homeostasis: integrated meta-analysis across five vertebrate species and functional validation in aquatic embryonic cells

Logan Germain, **Holly Mackay**, Taylor Bird, Lihua Xue & Louise M Winn

**Abstract included with flash talk abstracts*

P15. MATERNAL MAMMARY GLAND DEVELOPMENT IS ALTERED FOLLOWING PRENATAL CANNABIS SMOKE EXPOSURE IN MICE

Yiye Wang[*1], David Tovar-Parra[2], Tina Podinić[1], Maria Sunil[3], Andie MacAndrew[1], Isabelle Plante[2], Elyanne Ratcliffe[3], Sandeep Raha[1]

[*]Presenting Author
[1]Department of Pediatrics and Graduate Program in Medical Sciences, Faculty of Health Sciences, McMaster University, Hamilton, ON, Canada, [2]INRS, Centre Armand-Frappier Santé Biotechnologie, Laval, Québec, [3]Department of Pediatrics, Division of Gastroenterology and Nutrition, Farncombe Family Digestive Health Research Institute, McMaster University, Hamilton, ON, Canada

Background: Breast milk is essential for infant growth and development, providing nutrients and bioactive factors that shape early-life health. Breast milk composition depends on proper mammary gland (MG) differentiation during pregnancy, a critical window for its development. Maternal exposures to external insults during this period may disrupt post-natal MG function and compromise offspring health. The effects of gestational cannabis use, mostly through smoking, on the mechanisms behind MG development remain largely unexplored. We hypothesize that cannabis smoke disrupts MG development by altering hormonal and adipogenic signaling.

Methods: Pregnant CD-1 mice were exposed daily to smoke from six ∆9-tetrahydrocannabinol (THC)-dominant cannabis cigarettes (12-14% THC:0-2% CBD) or filtered air from embryonic day (E) 6.5 to

E18.5. MGs were harvested on post-natal day 21 and subjected to histological, gene and protein expression analyses.

Results: Trichrome Masson staining of exposed glands revealed morphological changes suggestive of altered lipid metabolism and accelerated development involution, characterized by the presence of brown adipose tissue and tissue showing post-lactation remodeling hallmarks. Transcript levels of adipogenic regulators (*Pparg*, *Adipoq*, *Irs1*) were significantly altered. Genes involved in milk lipid biosynthesis (*Scd1*, *Srebp1*) were downregulated while a key mediator of MG development, prolactin receptor was upregulated 1.3-fold. Among milk proteins, whey acidic protein (Wap) transcript levels decreased by 0.4-fold, whereas β-casein levels and both WAP and β-casein protein levels were not significantly impacted.

Conclusion: We demonstrate that gestational cannabis smoke exposure induces morphological and molecular changes associated with adipogenic regulation, lipid biosynthesis and prolactin signaling. These findings suggest that cannabis smoke exposure during pregnancy can alter the differentiation of the MG, potentially leading to significant consequences for lactation and neonatal health.

Funding: Department of Pediatrics, McMaster University: Core Builder Fund

P16. MicroRNAs AS PREDICTIVE BIOMARKERS: ASSESSING EMBRYO QUALITY AND ANEUPLOIDY

L. Lewicki[1], D.H. Betts[1,2]

[1]Department of Physiology and Pharmacology, [2]Department of Obstetrics and Gynaecology, Schulich School of Medicine and Dentistry, Western University, London, ON

Aneuploidy significantly affects embryo viability, making the selection of a single competent embryo crucial to minimizing the risk of gestation loss. Testing for aneuploidy in embryos aims to enhance implantation rates and increase pregnancy success for in vitro fertilization (IVF) patients. Despite advancements in pre-implantation genetic testing (PGT) through genomic analyses of biopsied cells and improved biopsy techniques, these methods remain invasive and controversial. This research project investigates alternative non-invasive genetic testing method for detecting aneuploidy in pre-implantation embryos. We specifically focus on utilizing microRNAs (miRNAs) released by pre-implantation embryos into the culture media as potential genetic markers for identifying incompetent embryos.

Mouse embryos at the 2-cell stage will be collected from gonadotropic super-ovulated female mice after mating. The embryos will be cultured to the 4-cell stage then treated with the AZ3146 to induce aneuploidy. AZ3146 is a monopolar spindle 1 kinase inhibitor that disrupts proper chromosome segregation during cell division. Subsequently, these aneuploid embryos will be cultured, and the miRNAs present in the culture media will be collected and assessed using RNA sequencing. Proportions of cell lineage markers will also be assessed using immunofluorescence confocal imaging.

We aim to understand the effect of aneuploidy on lineage marker expression as well as to identify distinct differences in the miRNA profiles of aneuploid and euploid embryos. This study seeks to establish a non-invasive approach to embryo assessment, potentially improving current practices in embryo selection and IVF outcomes.

Funding: Canadian Institutes of Health Research, Children's Health Research Institute (CIHR), Mitacs Accelerate

P17. Sex-driven coordination of oxidative stress and DNA damage responses in the murine fetal liver following *in utero* benzene exposure

Perri M. Grant[1], Frederick Stephenson[1], Megan Cull[1], Lihua Xue[1], Louise M. Winn[1,2]

[1]Dept. of Biomedical and Molecular Sciences, Queen's University Kingston, Ontario Canada, [2]School of Environmental Studies, Queen's University Kingston, Ontario Canada

Background and Objectives: Benzene is a widespread environmental carcinogen that crosses the placental barrier, resulting in fetal exposure during critical windows of development. Epidemiological and animal studies link *in utero* benzene exposure to increased childhood leukemia risk. The fetal liver, as the primary site of hematopoiesis during mid-gestation, is uniquely sensitive to redox imbalance and genotoxic stress. This study aimed to characterize sex-specific temporal patterns of transcriptional responses spanning metabolic activation, oxidative stress signaling, and DNA damage pathways in the fetal liver following *in utero* benzene exposure.

Methods: Pregnant CD-1 mice were administered 200 mg/kg benzene or vehicle (corn oil) via intraperitoneal injection on gestational days 8, 10, 12, and 14. Fetal livers were collected at 2, 6, and 24 hours after the final exposure. Relative gene expression of bioactivation (*Cyp2e1*, *Mpo*, *Nqo1*), oxidative stress response (*Nrf2*, *Hmox1*, *Sod2*), DNA repair (*Ogg1*, *Apex1*), and DNA damage signaling (*Atm*, *p53*, *p21*) was assessed by RT-qPCR with relative gene expression calculated using normalized and calibrated relative quantification (adapted ΔΔCq method).

Results: Benzene exposure produced temporally organized, male-driven transcriptional changes in the fetal liver. At 2 hours, *Cyp2e1* expression was significantly increased in benzene-exposed males relative to male controls, suggesting enhanced early bioactivation. By 24 hours, benzene-exposed males showed coordinated upregulation of *Nrf2*, *p21*, and *Apex1* compared to male controls, reflecting integrated activation of antioxidant defenses, cell cycle regulation, and DNA repair. Corresponding changes were not observed in females.

Conclusion: *In utero* benzene exposure induces a temporally coordinated, male-driven transcriptional response in the fetal liver. These findings highlight fetal sex as a key biological variable in toxicological response and offer mechanistic insight into sex-specific developmental disease risk.

Funding: CIHR, CAMCOO

P18. Plasma Viscoelastic Characterization of COVID-Associated Coagulopathy in Pregnancy at Delivery and Postpartum

*__Mustapha Alayan__[1,2] *Caroline Mwubaha[1,2], Maica Yunon[2,3], Yousra Tera[1], Maha Othman[1,3,5], Sandra Blitz[4], Deborah Money[4] and the CANCOVID-Preg Team

[1]Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada, [2]Healthcare Administration Program, St Lawrence College, Kingston, Ontario, Canada, [3]School of Baccalaureate Nursing, St Lawrence College, Kingston, Ontario, Canada, [4]Departments of Obstetrics and Gynecology, Medicine and the School of Population and Public Health, Faculty of Medicine, [5]Faculty of Medicine, Mansoura University, Mansoura, Egypt
*Equal contribution

Background and objectives: Pregnancy induces a hypercoagulable state that peaks at delivery and gradually resolves postpartum. COVID-19 introduces an additional prothrombotic stressor through endothelial dysfunction, increased thrombin generation, and fibrin-dominant clot formation. In pregnancy, this may alter peripartum coagulation dynamics. While whole blood viscoelastic testing is widely used, plasma-based thromboelastography (TEG) offers a practical alternative for multicentre studies. The plasma TEG phenotype of COVID-19 in pregnancy remains undefined. We aimed to characterize plasma coagulation dynamics at delivery and postpartum, isolating enzymatic and fibrin contributions independent of cellular elements.

Methods: This sub-study used samples from the CANCOVID-Preg national biorepository (2020–2022), spanning 9 Canadian provinces/territories. Fourteen pregnant individuals (age 31–42; BMI 21–24) with mild COVID-19 and no major comorbidities were included. Citrated plasma was collected at delivery and 4–8 weeks postpartum. Plasma TEG (TEG® 5000) assessed standard parameters (R, K, α-angle,

MA, CI) and thrombin generation metrics (MRTG, TMRTG). Comparisons were made across delivery, postpartum, and healthy non-pregnant controls (n=9) using oneway ANOVA with Bonferroni correction ($p<0.05$). An exploratory spiking experiment exposed control plasma to COVID-19 delivery plasma.

Results: Postpartum plasma demonstrated a consistent hypocoagulable shift versus delivery, with prolonged R and K and reduced α-angle, MA, CI, and MRTG, indicating delayed clot initiation, impaired propagation, and reduced clot strength. Patient-level analyses confirmed this trend across 6 paired patient cohort. Spiking experiments showed reductions in α-angle and MRTG, suggesting circulating COVID-associated plasma factors impair clot propagation, with partial and parameter-specific effects.

Conclusion: This first plasma TEG study in COVID-19 pregnancy identifies a persistent postpartum hypocoagulable phenotype, in contrast to the expected gestational hypercoagulability. Findings suggest COVID-associated plasma factors disrupt coagulation balance. Establishing plasma TEG reference ranges is needed to distinguish true hypocoagulability from physiological normalization.

Funding: Internal Funding- St Lawrence College

P19. IRE1/XBP1s signaling regulates human placental syncytiotrophoblast formation

Jahdiel DeNobrega[1], Stephen J. Renaud[1,2*], Patrick Lajoie[1,2*]

[1]Department of Anatomy and Cell Biology, The University of Western Ontario, London, Ontario, Canada, N6A 5C1, [2]Children's Health Research Institute, London Health Sciences Centre Research Institute, London, Ontario, Canada

Background: The placenta is essential for fetal development, mediating maternal-fetal nutrient and gas exchange and producing hormones critical for pregnancy success. These functions depend on the syncytiotrophoblast (STB), a multinucleated layer formed through differentiation and fusion of cytotrophoblasts (CTBs). Impaired STB formation contributes to pregnancy complications such as preeclampsia, a leading cause of maternal and infant mortality. The unfolded protein response sensor IRE1 regulates endoplasmic reticulum proteostasis, and mice lacking IRE1 exhibit failed placentation and embryonic lethality. However, its role in human trophoblast differentiation remains unknown. We hypothesized that IRE1 and its canonical effector, X-box binding protein 1 (XBP1), promote STB formation.

Methods: Human trophoblast stem (TS) cells were differentiated into STB over 5 days. Differentiation was assessed by measuring expression of CTB (*TEAD4*) and STB (*CGB*) markers, and by determining chorionic gonadotropin (hCG) levels. IRE1/XBP1 pathway activation was evaluated by XBP1 splicing and expression of downstream targets (*HSPA5*, *EDEM1, HERPUD1*). IRE1 was inhibited using STF-083010. CRISPR-mediated disruption of *XBP1* was performed by transducing cells with a construct delivering Cas9 and sgRNAs targeting exon 1, followed by puromycin selection. $P<0.05$ was considered statistically significant.

Results: TS cells effectively differentiated into STB, as indicated by decreased *TEAD4* and increased *CGB* expression, along with increased protein levels of hCG ($p<0.05$). IRE1 expression, XBP1 splicing, and expression of *HSPA5*, *EDEM1,* and *HERPUD1*, were increased during STB formation (all $p<0.05$), indicating robust IRE1 activation during syncytialization. Inhibition of IRE1 using STF-083010 impaired STB formation. CRISPR-mediated disruption of *XBP1* significantly reduced XBP1s protein levels, establishing an effective loss-of-function model. Ongoing studies are evaluating the impact of *XBP1* loss on STB formation.

Conclusion: IRE1/XBP1s signaling is a key regulator of STB formation and may represent a promising target to enhance STB formation in pregnancy complications such as preeclampsia.

P20. Longitudinal assessment of utero-placental vascular development in rat pregnancy

Jordan Jones [a 1], Megan Lave [a], Stephen Renaud [a, b]

[a] Department of Anatomy and Cell Biology, Schulich School of Medicine & Dentistry, University of Western Ontario, MSB428, 1151 Richmond Street, London, ON, N6A 5C1, Canada, [b] Children's Health Research Institute, London Health Sciences Centre Research Institute, London, ON, Canada

Introduction: Successful pregnancy depends on proper utero-placental vascularization, involving remodeling of uterine vessels into dilated, low-resistance structures that ensure adequate maternal blood flow to the placental exchange surface. In parallel, the placenta undergoes extensive branching morphogenesis to increase surface area for nutrient and gas exchange between maternal and fetal circulations. If these processes are impaired, adverse outcomes such as preeclampsia and fetal growth restriction can result. Rat models are widely used to study utero-placental vascularization due to their physiological similarities to humans, and ultrasound provides a non-invasive approach to assess vascular dynamics. Our objective was to characterize longitudinal changes in utero-placental vascularization across gestation in rats.

Methods: A Doppler ultrasound protocol was optimized to assess uterine artery hemodynamics in pregnant Wistar rats at gestational days (GD) 6.5, 12.5 and 18.5. Uterine artery structure was evaluated using histology to measure wall thickness, whole vessel and lumen size at the same timepoints. Maternal and fetal vascular spaces in GD 15.5 and 18.5 placentas were quantified following immunofluorescence for monocarboxylate transporter (MCT)1 and MCT4, which demarcate syncytialized cells lining maternal and fetal blood spaces, respectively. $P<0.05$ was considered statistically significant.

Results: As pregnancy progressed from GD 6.5 to E18.5, peak-systolic, end-diastolic and mean velocity progressively increased in uterine arteries ($P<0.05$), but there was no change in resistance or pulsatility index. Histological analysis showed no significant changes in uterine artery lumen size or wall thickness between GD 6.5 and 18.5. In the placenta, fetal vascular area and perimeter increased from GD 15.5 to 18.5 ($P<0.05$) while maternal vascular area decreased ($P=0.01$).

Discussion: These findings highlight distinct vascular remodeling patterns during normal rat pregnancy, with modest uterine artery structural changes and pronounced placental vascular adaptations. This approach provides insight into normal vascular development in rat pregnancy and a foundation for studying pathological pregnancy.

P21. (FT3) Platelet GPIbα Dysfunction Is Associated with Abnormal Placental Structure and Adverse Pregnancy Outcomes

Vrisha Shah[1], Harmanpreet Kaur[1], Maha Othman[1,2,3]

[1]Department of Biomedical and Molecular Sciences, School of Medicine, Queen's University, Kingston, Ontario, Canada; [2]School of Baccalaureate Nursing, St Lawrence College, Kingston, Ontario, Canada; [3]Clinical Pathology Department, Faculty of Medicine, Mansura University, Egypt

**Abstract included with flash talk abstracts*

P22. (FT4) Enhancing Early Obstetric Ultrasound Competency in Medical Education: A Resident-Led POCUS Workshop Evaluation

Natalie Kearn*[1], Hannah Brown*[1], Danielle Campagnolo[2], **Fariya Zaheer**[1], Annalise Gignac[1], Nooh Kabir[1], Stanley Chen[1], Aurelia Zhou[1], Joseph Newbigging[1]
*these authors contributed equally

[1]Department of Emergency Medicine, Faculty of Health Sciences, Queen's University, Kingston, Ontario, Canada, [2]Department of Obstetrics & Gynecology Faculty of Health Sciences, Queen's University, Kingston, Ontario, Canada

**Abstract included with flash talk abstracts*