BREAKTHROUGHS IN REPRODUCTION AND DEVELOPMENT

NOUVELLES AVANCÉES EN REPRODUCTION ET DÉVELOPPEMENT

Research Day

Centre for Research in Reproduction and Development (CRRD) at McGill

Tuesday, May 31, 2016

McGill New Residence Hall
3625 Avenue du Parc
Montréal, Québec
**BREAKTHROUGHS IN REPRODUCTION AND DEVELOPMENT**

*Research Day 2016*

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| 9:15-10:00    | Trainee oral presentations (Chairs: Fatima Tokhmafshan and Océane Albert)  
| **O-01. Amanda Baumholtz**, “Claudins Regulate Cell Shape and Localization of Signaling Proteins at the Apical Cell Surface During Neural Tube Closure”  
| **O-02. Alex Yu**, “Peroxiredoxins Protect Spermatozoa Against Oxidative Damage and Regulate Capacitation”  
| **O-03. Aaron Kwong**, “Basal F-Actin Network Facilitates Rosette Reorganization in Primed Pluripotent Stem Cells during Mouse Blastocyst Implantation” |
| 10:00-10:45   | **Dr. Robert S. Viger**, Centre Hospitalier de l'Université Laval (CHUL), “Understanding gonadal development and function: what we can learn from transcription studies” – introduced by Dr. Cristian O’Flaherty |
| 10:45-11:00   | Health Break / Briefing of Judges                                      |
| 11:00-11:45   | **Dr. Jodi Anne Flaws**, Department of Comparative Biosciences, University of Illinois, “The Effects of Endocrine Disrupting Chemicals on Female Reproduction” – introduced by Dr. Sarah Kimmins |
| 11:45-12:45   | Lunch                                                                 |
| 12:50-14:20   | Poster Session                                                          |
| **P-01. Sunghoon (Daniel) Lee**, “In Utero Exposure to DEHP Alters Steroidogenesis in the Adult Male Offspring: Reversibility of the Effects Using Pharmacological Means”  
| **P-02. Yining Li**, “Smad4 and Foxl2 are Required for FSH Synthesis in adult mice”  
| **P-03. Sathvika Venugopal**, “Identification of the Source of Cholesterol Used for Steroid Biosynthesis in Primary Rat Leydig Cells”  
| **P-04. Gauthier Schang**, “A Novel Role for Class I Histone Deacetylases as Positive Regulators of Fshβ-Subunit Expression in Gonadotrope Cells”  
| **P-05. Chirine Toufaily**, “Evolution of the Mammalian Gnrh Receptor May Enhance Gonadotropin Regulation by Gnrh”  
<p>| <strong>P-06. Luisina Ongaro Gambino</strong>, “Re-Examining Activin B’S Role in Follicle-Stimulating Hormone Synthesis in Vivo” |</p>
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P-37. Cynthia Franci, “In Ovo Potency of Polycyclic Aromatic Hydrocarbons (Pahs) In Chicken Embryos”


P-39. Han (Aileen) Yan, “A Comparison of the Effects of Brominated Diphenyl Ether-47 (BDE-47) and Isopropylated Triphenyl Phosphate (IPPP) on Murine Limb Development in Vitro”

14:30-15:15 Trainee oral presentations (Chairs: Kai Sheng and Chirine Toufaily)

O-04. Marc-Olivier Turgeon, “Development of a Novel, Crispr-Cas9 Derived Mouse Model for Studies of Igsf1 Deficiency Syndrome”

O-05. Nazem El Hussein, “Exposure of Organogenesis-Stage Embryos to Hydroxyurea Alters the Expression of P53-Family Related Genes that are Involved in Limb Development”


15:15-16:00 Dr. Sally A. Camper, Department of Human Genetics, University of Michigan Medical School, “Combined Pituitary Hormone Deficiency: gene networks and stem cells” – introduced by Dr. Aimee Ryan

16:00-16:10 Concluding Remarks: Dr. Daniel Bernard

16:10-17:00 Awards Presentation & Reception / Take down posters
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Journée de recherche 2016
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le mardi 31 mai 2016
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Inscription et café / Installation des affiches

9 h  
Mot de bienvenue : Dr. Daniel Bernard

9 h 15  
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Pause santé / Briefing des juges

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11 h 45  
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12 h 50  
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16 h 00  Mot de conclusion : Dr. Daniel Bernard

16 h 10  Présentation des prix, réception et démontage des affiches

17 h 00  Au revoirs!
UNDERSTANDING GONADAL DEVELOPMENT AND FUNCTION: WHAT WE CAN LEARN FROM TRANSCRIPTION STUDIES

Defining how genes get turned on and off in a correct spatiotemporal manner is essential for our understanding of biological processes in both health and disease. Our research is focused on elucidating how GATA proteins, and in particular GATA4, contribute to proper gonadal function and how these actions are regulated. GATA4 belongs to a family of proteins known as GATA factors (named for the DNA sequence that they bind) that function as essential regulators of gene transcription in multiple organ systems. In mammalian reproductive tissues, GATA4 is likely the earliest factor required to initiate development of the urogenital ridge. While its expression is not restricted to either ovarian or testicular somatic cells, GATA4 is nonetheless later required for gonadal sex differentiation in both sexes. GATA4 is also essential for normal male and female fertility in the adult gonads where it controls a variety of key processes such as follicle growth, steroidogenesis, and blood-testis barrier integrity. The identification of GATA4-dependent functions in the gonads has been made possible by different gonad-specific knockout models that have directly focused on the GATA4 transcription factor. These studies, however, have been much less informative about defining its mechanism of action in gonadal cells—that is: what are its specific target genes and how does it orchestrate gene expression during early development and later during adulthood? We have used a combination of both in vitro and in vivo promoter characterization studies to help provide some answers. While GATA4 is not exclusive to the gonads, its transcriptional regulatory partners are most often specific to gonadal cells. This unique combinatorial code of transcription factors, which hinge on the presence of GATA4, is responsible for coordinating the expression of several gonadal genes. GATA4 also possesses unique regulatory properties. Its gene is controlled by multiple promoters that are independently regulated to ensure that abundant GATA protein is produced. GATA4 activity itself is modulated by different post-translational modifications such as phosphorylation that is regulated by signaling pathways activated by pituitary gonadotropins. The role of GATA4 phosphorylation as well as the impact of direct GATA4-binding to its proposed target genes is currently being evaluated using both classic mouse transgenic models and well as by newer CRISPR-Cas9 gene editing techniques. These results of these and other ongoing studies, designed to address GATA4 regulation and mechanism of action, will be presented.

Brief Bio: Dr. Robert Viger received his B.Sc. (Hons.) degree in Biochemistry (1989) and Ph.D. in Pharmacology and Therapeutics (1995) from McGill University. He obtained his postdoctoral training in molecular endocrinology at the Clinical Research Institute of Montreal and began his own research group in the Department of Obstetrics, Gynecology, and Reproduction at Laval University as Assistant Professor in 1999. Viger was promoted through the academic ranks to full Professor in 2009. Since 1997, he is also a Senior Scientist at the Centre Hospitalier Universitaire (CHU) de Quebec Research Centre where his laboratory is housed. He is currently the leader of the male reproductive biology theme of the Quebec Reproduction Network (RQR) and in 2014, he was appointed as Scientific Director of Laval University’s Centre for Research in Reproduction, Development, and Intergenerational Health (CRDSI). Viger leads an active research program studying the molecular biology of gonadal development and function.
Bisphenol A (BPA) is a high production volume chemical used in a variety of consumer products. Most notably, BPA is present in polycarbonate plastics, the epoxy resin liners of aluminum cans, and thermal receipts. BPA has been detected in human urine, blood, breast milk, amniotic fluid, and ovarian follicular fluid. This is of concern because previous epidemiological and experimental studies indicate that BPA is an endocrine disrupting chemical. Further, previous experimental studies indicate that prenatal BPA exposure causes several adverse developmental effects in the F1 offspring, including subfertility. Few studies, however, have investigated whether prenatal BPA exposure has transgenerational effects on the developing ovary and female fertility. Thus, we tested the hypothesis that prenatal exposure to BPA inhibits the process of germ cell nest breakdown, a critical process for normal ovarian development, in the F1, F2, and F3 generations of mice. Further, we tested the hypothesis that prenatal exposure to BPA reduces female fertility in the F1, F2, and F3 generations of mice. Our data indicate that prenatal exposure to BPA inhibits germ cell nest breakdown in the F1 generation involves an ability of BPA to inhibit apoptosis in the developing ovary. Further, our data indicate that prenatal exposure to BPA reduces female fertility in the F1, F2, and F3 generations of mice. Collectively, our studies indicate that prenatal exposure to BPA has transgenerational effects on female fertility, but not germ cell nest breakdown in mice. This work was supported by NIH P01 ES022848, EPA RD-83459301, and NIH T32 ES 007326.

Brief Bio: Dr. Jodi Flaws received her B.Sc. in Biology (1986) from St. Xavier University in Illinois, her M.Sc. in Biology (1989) from Loyola University, also in Illinois, and her Ph.D. in Physiology (1994) from the University of Arizona. She obtained her postdoctoral training at Johns Hopkins in the Division of Reproductive Biology and at the University of Maryland in the Women’s Health Research Group. She was appointed Assistant Professor in the Department of Epidemiology and Preventive Medicine at the University of Maryland in 1997 and was promoted to Associate Professor in 2001. From 2002-2006, she was Division Head for the Division of Gender-Based Epidemiology. In 2006, she moved to the Department of Comparative Biosciences at the University of Illinois where she is full Professor. Flaws’ research group focuses on determining the genetic and environmental factors that affect the female reproductive system.
Congenital hypopituitarism occurs approximately 1/4000 births and can be life threatening if not identified and treated effectively. About a dozen different genetic causes have been reported, but most of the patients have no molecular diagnosis. Mutations in the transcription factors POU1F1 and PROP1 cause recessive combined pituitary hormone deficiency (CPHD) with no additional abnormalities, while mutations in several of the other causal genes exhibit dominant inheritance with incomplete penetrance and are associated with variable clinical features, sometimes including craniofacial and eye defects. Mutations in PROP1 are the most common known genetic cause of CPHD, and the course of disease is progressive, sometimes leading to life threatening adrenal insufficiency. Our objectives are to understand the role of PROP1 in disease pathophysiology and to improve molecular diagnosis of CPHD. To accomplish these goals, we compared gene expression profiles in developing pituitary glands and pituitary stem cell derived colonies from Prop1 and Pou1f1 mutant mice, and we performed chromatin immunoprecipitation (ChIP-Seq) experiments to identify direct downstream targets of PROP1. We discovered that Prop1 deficiency has profound effects on stem cell behavior. The Prop1 specific changes in gene expression, combined with the knowledge of genes directly bound by PROP1, revealed the underlying mechanistic genetic network. Prop1 suppresses expression of genes involved in tight junction maintenance, promoting progenitor release from the stem cell niche. It also activates expression of matrix metalloproteinases, several signaling pathways including BMP, WNT, NOTCH, and hedgehog, and transcription factors that drive epithelial to mesenchymal transition in other systems. We conclude that PROP1 normally promotes the transition of pituitary stem cells to differentiating cells by driving an epithelial to mesenchymal like transition process. Our findings extend our understanding of pituitary development and the mechanism of PROP1 action, and provide intriguing candidate genes for CPHD of unknown etiology.

Brief Bio: Dr. Sally Camper received a B.A. degree in Chemistry from University of Delaware and a Ph.D. in Biochemistry from Michigan State University. She completed her postdoctoral training at Fox Chase Cancer Research Institute, Philadelphia, PA and at Princeton University, NJ and was among the pioneers in the use of transgenic mice to study developmentally regulated gene expression. She joined the faculty of Human Genetics at UM in 1988 and served as Dept. Chair for 11 years (2005-2015). Camper is recognized for research in the genetics of birth defects, especially neuroendocrine, hearing, and skeletal developmental disorders. Her studies with human patients and genetically modified mice have revealed genetic causes of disease, pathophysiological mechanisms, and potential therapeutics. Camper is devoted to educating biomedical science researchers and received a mentoring award from the University of Michigan Rackham Graduate School and the Endocrine Society. She was a founder and Associate Director of the graduate Program in Biomedical Sciences, a common gateway for Ph.D. studies in 14 disciplines. She also founded and directed the Transgenic Animal Model Core for over 2 decades. She has held leadership positions in the Genetics Society of America, the American Society of Human Genetics, the Endocrine Society, and the International Mammalian Genome Society. She has also served on the National Genome Research Institute Board of Scientific Counselors and has experience on numerous advisory panels, study sections and editorial boards.
ORAL PRESENTATIONS
Morphogenetic remodeling of the neural plate into a closed neural tube requires synchronization of cell shape changes with cell movements within the neural and non-neural ectoderm. Throughout neural tube closure, the integrity of these epithelial cell layers is maintained by intercellular junctions, the most apical of which are tight junctions. We discovered that members of the claudin family of integral tight junction proteins regulate molecular and morphological changes that are essential for both early and late events during neural tube morphogenesis. Claudins regulate paracellular permeability, apical-basal cell polarity and cell adhesion, and link the tight junction to the actin cytoskeleton. Removal of Claudin-3, -4, and -8 from tight junctions of the chick ectoderm using the C-terminal domain of Clostridium perfringens enterotoxin (C-CPE) resulted in folate-resistant neural tube defects in 100% of treated embryos that are caused by defective apical constriction and convergent extension. Open neural tube defects were also observed in mouse embryos treated with C-CPE. Removal of Claudin-3 from the non-neural ectoderm using a Claudin-3-specific C-CPE variant affected only the final phase of epithelial remodeling that joins the apposed neural tube folds to form a closed tube. Molecular analyses revealed that apical-basal polarity was maintained. However, apical accumulation and/or localization of Rho-GTPase and PCP signalling components were dramatically reduced/altered. We hypothesize that the cytoplasmic tails of claudins uniquely interact with components of Rho-GTPase signalling and polarity complexes to coordinately regulate changes in cell movements and cell shape that are required for neural tube closure.

Funding sources: Supported by NSERC
PEROXIREDOXINS PROTECT SPERMATOZOA AGAINST OXIDATIVE DAMAGE AND REGULATE CAPACITATION

Alex Yu¹,², Donghyun Lee¹, Adel R. Moawad¹,³, Cristian O’Flaherty¹,²,³,⁴

¹Urology Research Laboratory, Research Institute-MUHC, Montréal, Québec, Canada
²Department of Experimental Medicine, McGill University, Montréal, Québec, Canada
³Department of Experimental Surgery, McGill University, Montréal, Québec, Canada
⁴Department of Pharmacology and Therapeutics, McGill University, Montréal, Québec, Canada

Introduction and aims: Infertility affects one in six couples and in half of these cases, male factors are to blame. Spermatozoa need to undergo a series of events called capacitation to achieve fertilizing ability. Low levels of reactive oxygen species (ROS), increase of tyrosine phosphorylation and of actin polymerization are associated with capacitation. Peroxiredoxins (PRDXs) are enzymes that protect cells against oxidative stress and modulate ROS signaling. The aim was to study the role of PRDXs in the antioxidant protection and redox signaling regulation to assure sperm viability and capacitation.

Experimental Approach: Percoll-selected spermatozoa from healthy donors were incubated with MJ33 (specific PRDX6 PLA₂ activity inhibitor), or thiostrepton (2-Cys PRDX peroxidase activity inhibitor). Sperm viability and motility were determined by hypo-osmotic swelling test and CASA, respectively. Sperm capacitation was induced by fetal cord serum ultra-filterate and monitored by the ability of spermatozoa to undergo acrosome reaction, tyrosine phosphorylation or actin polymerization. Lipid peroxidation was measured by BODIPY C11 fluorescence.

Results: TSP and MJ33 increased sperm lipid peroxidation. MJ33 impaired sperm viability in a dose dependent manner, and this effect was exacerbated by H₂O₂. D-Penicillamine prevented the reduction of sperm viability when H₂O₂ was present with thiostrepton but not with MJ33. Acrosome reaction, tyrosine phosphorylation and actin polymerization were prevented at 5-20 μM TSP or MJ33.

Conclusions: PRDXs protect spermatozoa against oxidative stress, assuring normal sperm function. PRDX6 PLA₂ activity is important to assure survival of spermatozoa. Further studies are necessary to elucidate the mechanisms of survival regulated by PRDXs.

Funded by CIHR
ORAL 3

BASAL F-ACTIN NETWORK FACILITATES ROSETTE REORGANIZATION IN PRIMED PLURIPOTENT STEM CELLS DURING MOUSE BLASTOCYST IMPLANTATION

Kwong A.1,2, Honma-Yamanaka N.2, Yamanaka Y.1,2

1 Department of Human Genetics, McGill University
2 Goodman Cancer Research Centre, McGill University

The naïve-primed pluripotency transition marks one of the earliest cell commitments to specialization in early embryogenesis. The naïve state, represented by mouse embryonic stem cells (mESCs) and epiblast cells in the blastocyst, develop into the primed state, represented by mouse epiblast stem cells (EpiSCs) and epiblast in an egg cylinder embryo. While it has been previously demonstrated that the MAPK/ERK pathway is necessary for the naïve-primed transition in 2D cultures, we ask what relevancy this pathway has in 3D culturing for primed pluripotent rosette formation and by what mechanism do these cells reorganize into a rosette. After developing an optimized 3D culturing system for investigating the naïve-primed transition in vitro from single cell naïve mESC, our findings suggest the formation of a basal F-actin network helps facilitate proper reorganization from disordered clusters into canonical rosettes. Interestingly, as a consequence of basal f-actin stress, rampant bleb formation is demonstrated during reorganization. Culturing cells with PD-0325901 (MEK inhibitor), Y-27632 (stress fiber inhibitor), and RGDS peptide (β-integrin antagonist) revealed that while rosette development persisted, disorganized cluster formation was significantly increased and cyst formation was nearly completely arrested. Indeed, live imaging GPI::GFP ESC constructs in the presence PD-0325901 showed cluster reorganization incompetence. Our findings suggest that the basal F-actin network plays a crucial role in morphological reorganization into a rosette and consequently cyst development as seen in embryonic development from inner cell mass reorganization into formation of the amniotic cavity in the egg cylinder embryo.

Funding: CIHR, NSERC, CRRD, McGill Fac Med
**DEVELOPMENT OF A NOVEL, CRISPR-CAS9 DERIVED MOUSE MODEL FOR STUDIES OF IGSF1 DEFICIENCY SYNDROME**

M-O. Turgeon\textsuperscript{1,2}, T. L. Silander\textsuperscript{2,3}, L. Ongaro\textsuperscript{1}, D. J. Bernard\textsuperscript{1,2,3}

\textsuperscript{1}Department of Anatomy and Cell Biology, \textsuperscript{2}Department of Pharmacology and Therapeutics, \textsuperscript{3}Integrated Program in Neuroscience, McGill University, Montreal, Canada

Loss-of-function mutations in the X-linked immunoglobulin superfamily, member 1 (\textit{IGSF1}) gene cause central hypothyroidism. IGSF1 is a transmembrane glycoprotein of unknown function. It is expressed in thyroid-stimulating hormone (TSH) producing thyrotrope cells of the anterior pituitary gland. The protein is co-translationally cleaved into N- and C-terminal domains (NTD and CTD). The CTD is trafficked to the plasma membrane, whereas the NTD is retained in the endoplasmic reticulum. Most intragenic \textit{IGSF1} mutations in patients map to the CTD. To better understand IGSF1 function, we used the CRISPR-Cas9 system to introduce a loss of function mutation into the IGSF1-CTD in mice. The modified allele harbors a 312 bp deletion, removing part of exon 18 and intron 18. The resulting mRNA is expressed, though at reduced levels relative to wild-type, and contains a novel, hybrid exon. This exon introduces frame-shift and a premature stop codon, which prevents the CTD from trafficking to the plasma membrane. \textit{Igsf1}\textsuperscript{Δ312/y} males are centrally hypothyroid. Though their serum thyroid-stimulating hormone (TSH) levels appear normal on a regular diet, pituitary expression of the TSH subunits, \textit{Tshb} and \textit{Cga}, is reduced relative to wild-type littermates. Hypothalamic thyrotropin-releasing hormone (TRH) stimulates TSH release. TRH receptor (\textit{Trhr}) mRNA levels are reduced in \textit{Igsf1}\textsuperscript{Δ312/y} males, suggesting that pituitaries of these animals may be less sensitive to TRH than wild-type mice. Indeed, in response to increased endogenous or exogenous TRH, \textit{Igsf1}\textsuperscript{Δ312/y} mice release significantly less TSH than wild-type mice. These results suggest that IGSF1 regulates pituitary sensitivity to TRH via regulation of TRHR expression.

Funding sources: CIHR operating grant MOP-133557 to DJB; NSERC CGS-M training grant awarded to MOT.
EXPOSURE OF ORGANOGENESIS-STAGE EMBRYOS TO HYDROXYUREA ALTERS THE EXPRESSION OF P53-FAMILY RELATED GENES THAT ARE INVOLVED IN LIMB DEVELOPMENT

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Hydroxyurea (HU), an anticancer agent, is a model teratogen used to study the embryonic stress response during organogenesis. Embryos exposed to HU on gestational day (GD) 9 have severe limb and tail malformations. Previously, we showed that HU treatment on GD9 significantly activated the P53 signalling pathway. P53 and related family proteins, P63 and P73, are implicated in limb development; absence of Trp53 sensitizes limbs to teratogenic insult while loss of Trp63 or Trp73 results in severe skeletal deformities. Here we investigated the impact of HU exposure on the expression of P53-family related genes that are involved in limb development. Saline or HU (400 or 600 mg/kg) was administered intraperitoneally to CD-1 mice on GD9; dams were euthanized 3 h later, embryos extracted and gene expression was analyzed. Ingenuity Pathway Analysis™ revealed that among the transcripts with altered expression 42 genes were causally associated with morphogenesis of bones and limbs, sixteen of which were associated with the P53 protein family. We examined the expression of four candidate genes: Ptch1, a cell surface receptor that is essential for proper digit formation and associated with preaxial polydactyly; Pax9, purportedly involved in distal limb development; Tbx5 involved in forelimb identity; and Jag2, associated with syndactyly. qRT-PCR analysis showed that Ptch1, Pax9 and Tbx5 were downregulated, while Jag2 was significantly upregulated. Together, these data suggest that P53 family proteins may play a role in mediating the effects of HU on the expression of several key genes that are important in limb development.

Funded by CIHR MOP-57867. NELH is the recipient of an award from CIHR-REDIH.
EFFECTS OF IN UTERO AND LACTATIONAL EXPOSURE TO NEW GENERATION “GREEN” PLASTICIZERS IN THE MALE RAT: A COMPARATIVE STUDY WITH DIETHYLHEXYL PHTHALATE (DEHP)

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*equal contribution

There is a critical need for innocuous plasticizer alternatives to replace phthalates due to the well-established anti-androgenic actions of these chemicals on male reproductive functions. Twenty candidate molecules were developed by McGill University's Department of Chemical Engineering to mimic the plasticity properties of DEHP; two compounds, 1,4-butanediol dibenzoate (BDD) and dioctyl succinate (DOS), that displayed the most innocuous profiles after in vitro testing on multiple cell lines were selected for in vivo studies. We hypothesized that BDD and DOS exert fewer endocrine disrupting effects than DEHP on the male rat reproductive system. Nine groups of Sprague Dawley timed pregnant rats were dosed daily with corn oil, BDD or DOS (30 or 300 mg/kg/day) from gestational day 8 to postnatal day 21 (PND21); DEHP and 1,2-cyclohexane dicarboxylic acid diisononyl ester (DINCH, currently used as a replacement for DEHP) served as reference compounds. In accordance with the literature, exposure to the higher dose of DEHP caused a significant decrease in anogenital index at PND3, providing evidence of a decreased exposure to androgens during fetal life, as well as an increased incidence of hemorrhagic testes at PND8. Neither of the new generation “green” plasticizers showed any significant effect on the organ weights, age or weight at puberty, or on serum testosterone levels in the male offspring. While further experiments are needed to fully assess the safety profile of our candidate compounds, the evidence obtained to date indicates that the endocrine disruptive properties of existing phthalates such as DEHP can be circumvented.

Funding sources: Supported by the CIHR Institute of Human Development, Child and Youth Health, the CIHR Training Program in Reproduction, Early Development, and the Impact on Health, and the Fonds de la Recherche du Québec en Santé.
# Poster Session Schedule

**CRRD Research Day 2016**  
**BREAKTHROUGHS IN REPRODUCTION AND DEVELOPMENT**  
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**Please Note:** Awards will be given for the top presentation, from member labs, in each category: MSc, PhD and PDF. The following posters are available for viewing, between 12:45 and 14:30, but will not be judged because they either do not fall in one of the three categories or are from non-member labs. We thank ALL presenters for participating!

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P-35 Bell, Michael (Chan)
Di-(2-ethylhexyl) phthalate (DEHP) is one of the most abundantly produced plasticizers used in the production of polyvinyl chloride products and can be found in food products, cosmetics, furniture, and medical equipment. DEHP has been detected in umbilical cord blood and amniotic fluid suggesting exposure begins early in development.

Previous work in the rat model has shown in utero exposure to > 100 mg DEHP/kg/d results in decreased levels of serum testosterone and aldosterone at postnatal day (PND) 60. Furthermore, this decrease in aldosterone was retained in the elderly rat (PND200). Interestingly, short-term administration of a low-salt diet, a stimulus for aldosterone secretion, resulted in increased aldosterone levels similar to controls, suggesting that the zona glomerulosa retained its steroidogenic response to circulating sodium levels.

The goal of the current study was to ascertain whether DEHP alters the steroidogenic capacity of cells to produce testosterone and corticosterone in response to hormone stimulation. We treated pregnant dams with oil, 1, or 300 mg DEHP/kg/d from gestational day (GD) 14 until birth. At PND60, male offspring were injected with human chorionic gonadotropin (hCG; 100 I.U.), or adrenocorticotropic hormone (ACTH; 500 µg/kg) one hour prior to blood collection. Serum analysis revealed that DEHP exposure alters basal testosterone and corticosterone levels. Treatment of these animals with supraphysiological concentrations of hCG or ACTH resulted in steroid levels similar to that of hormone-stimulated controls suggesting the endocrine-disrupting effects of DEHP in the testes and adrenals can be reversed by pharmacological means.

Funding sources: Supported by CIHR grant MOP-111131 and a CRC
SMAD4 AND FOXL2 ARE REQUIRED FOR FSH SYNTHESIS IN ADULT MICE

Yining Li¹, Ying Wang¹, Ulrich Boehm², Alexandre Boyer³, Derek Boerboom³, and Daniel J. Bernard¹

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²Department of Pharmacology and Toxicology, University of Saarland School of Medicine, Homburg, Germany
³Département de Biomédecine vétérinaire, Université de Montréal, St-Hyacinthe, Quebec, Canada

Pituitary follicle-stimulating hormone (FSH) is an essential regulator of fertility in females and of quantitatively normal spermatogenesis in males. Activins directly stimulate FSH synthesis by regulating transcription of the FSHβ subunit gene (Fshb) in gonadotrope cells. Activins signal via SMAD4 and the transcription factor FOXL2 to regulate Fshb. Gonadotrope-specific deletion of Smad4 and Foxl2 using Cre-lox produces mice that are profoundly FSH-deficient and, in the case of females, sterile. However, the Cre allele used in this model is first active during pituitary development (dpc12.75). Thus, it is unclear whether the diminished FSH synthesis reflects impaired gonadotrope development or the necessity for SMAD4 and FOXL2 in adulthood. To discriminate between these possibilities, we generated a new mouse line that enables the inducible deletion of Smad4 and Foxl2 in gonadotropes of adult mice (hereafter S4F2icKO). Prior to treatment, females showed normal puberty onset, estrous cyclicity, and fertility. At 10 weeks, mice were treated with tamoxifen to induce Cre activity or with oil vehicle. S4F2icKO females became sterile after induced recombination. Tamoxifen itself did not affect fertility in females with the control genotype. In S4F2icKO males, pituitary Fshb mRNA levels were reduced to 30% of control values. Although not yet completed, our preliminary data suggest that FOXL2 and SMAD4 are required for FSH synthesis in gonadotrope cells of adult mice.

Funding resources: This research was funded by CIHR MOP-133394 to D.J.B. Y. Li is supported by a Samuel Solomon in Endocrinology Award.
POSTER 3

IDENTIFICATION OF THE SOURCE OF CHOLESTEROL USED FOR STEROID BIOSYNTHESIS IN PRIMARY RAT LEYDIG CELLS

Sathvika Venugopal\textsuperscript{1}, Daniel B. Martinez-Arguelles\textsuperscript{1}, and Vassilios Papadopoulos\textsuperscript{1}

\textsuperscript{1}Research Institute of the McGill University Health Centre and Department of Medicine, McGill University, Montreal, Quebec, H4A 3J1, Canada.

Hormone sensitive acute steroid biosynthesis is initiated when cholesterol from intracellular sources in trafficked to the inner mitochondrial membrane (IMM) where it is converted to pregnenolone by the cytochrome P450 side chain cleavage enzyme (CYP11A1). Despite the numerous studies carried out, the exact source of cholesterol as well as the mechanism by which it is transported to IMM remains to be elucidated. D4 is the fourth domain of Perfringolysin O protein and has the ability to bind cholesterol-enriched cellular membranes. D4 expressed in steroidogenic MA-10 mouse tumor Leydig cells primarily localized the inner leaflet of plasma membrane (PM), but upon 30-60 minutes treatment with dibutyryl-cAMP (dbcAMP), a significant dissociation from PM labeling was observed. D4 was further employed in this study to understand the source organelle for cholesterol in isolated primary rat Leydig cells during acute steroidogenesis. mCherry-D4 was packaged into lentiviruses using the Lenti-X packaging system (Clontech). A high titer of about $10^8$ infectious units (ifu)/ml of lenti-viruses were obtained. Purified Leydig cells were obtained from 64-day-old Sprague-Dawley rat testes by Percoll gradient centrifugation. The cells were plated and infected with concentrated lentiviral particles containing mCherry-D4. 24 hours post infection, over 95% of the primary rat Leydig cells were found to express the mCherry-D4. In a fraction of these cells fluorescence localized at the PM. Upon dbcAMP stimulation a significant reduction in PM labeling was observed within 60-90 minutes, suggesting that PM is a source of cholesterol used for steroid hormone formation in primary rat Leydig cells.

Funding sources: CIHR grant MOP-102647 and CRC.
A NOVEL ROLE FOR CLASS I HISTONE DEACETYLASES AS POSITIVE REGULATORS OF FSHβ-SUBUNIT EXPRESSION IN GONADOTROPE CELLS

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¹ Department of Pharmacology and Therapeutics, McGill University, Montreal, Quebec, Canada

Hypothalamic GnRH and intra-pituitary activins are regarded as the primary drivers of FSH synthesis and secretion by pituitary gonadotrope cells. Both regulate transcription of the FSHβ subunit (Fshb), although their underlying mechanisms of action are incompletely described. One recent model suggested that the murine Fshb promoter is tonically repressed by histone deacetylases (HDACs). Both GnRH and trichostatin A (TSA, an HDAC inhibitor) stimulated HDAC nuclear export and Fshb expression in LβT2 cells (an immortalized murine gonadotrope-like cell model). However, we have been unable to replicate these findings. Specifically, after treating LβT2 cells with activin A and/or TSA (331 nM) for 6 h, we observed a robust decrease rather than increase in both basal and activin A-stimulated Fshb mRNA levels. We repeated these analyses with lower concentrations of TSA (45 nM or 25-fold greater than its IC50) in both LβT2 cells and murine primary pituitary cultures and observed similar inhibition of Fshb. TSA inhibits both class I and class II HDACs. To determine the relative roles of these proteins in gonadotropes, we treated murine primary pituitary cells with class I (entinostat) or class II (MC158 or TMP269) specific inhibitors at concentrations of 42.5 µM, 2.5 µM, and 3.9 µM respectively (i.e. 25-fold their respective IC50s). Like TSA, entinostat specifically inhibited basal and activin A-induced Fshb expression. Neither class II inhibitor affected Fshb. Collectively, these data suggest a previously unappreciated and unanticipated role for class I HDACs as positive regulators of FSH synthesis in cultured murine gonadotrope cells. We are currently investigating their specific mechanistic roles.

Funding sources: supported by FRQS (GS), CIHR MOP 133394 (DJB)
The mammalian GnRH receptor (GnRHR) is unusual among G protein coupled receptors (GPCRs) in lacking an intracellular C-terminus (C-tail). The C-tail in many GPCRs mediates agonist-induced desensitization and receptor internalization. It was previously hypothesized that the loss of the GnRHR C-tail during mammalian evolution may have allowed for the generation of protracted luteinizing hormone (LH) surges in response to the preovulatory GnRH surge. To understand the functional significance of the loss of the C-tail, we generated knock-in mice in which the chicken GnRHR C-tail was fused in-frame to the C-terminus of the endogenous murine GnRHR (hereafter Gnrhr<sup>Ctail/Ctail</sup>). Contrary to our <i>a priori</i> prediction, neither serum LH nor pituitary luteinizing hormone beta subunit (Lhb) mRNA levels differ between adult Gnrhr<sup>Ctail/Ctail</sup> and wild-type males or females. In contrast, both serum FSH and pituitary follicle-stimulating hormone beta subunit (Fshb) expression are decreased in Gnrhr<sup>Ctail/Ctail</sup> males, but not in females on metestrus. Following bilateral gonadectomy, pituitary gonadotropin subunit mRNA levels as well as serum LH and FSH increase in males and females of both genotypes; however, the responses are blunted in Gnrhr<sup>Ctail/Ctail</sup> mice. Gnrhr<sup>Ctail/Ctail</sup> females exhibit abnormal estrous cyclicity and are subfertile. Reduced litter sizes could derive from impaired FSH-induced follicle maturation and/or from altered LH surge dynamics. Collectively, these observations suggest that the addition of a C-tail creates a signalling bias that preferentially hinders GnRH regulation of FSH. Based on the data collected thus far, we propose that the loss of the C-tail in mammalian GnRH evolution conferred a selective advantage by enhancing GnRH’s regulation of FSH rather than by enabling the LH surge.

Funding sources: FQRNT Team Grant PR-174948 and CIHR MOP-123447 to DB, and CRRD postdoctoral fellowship to CT.
Activins were discovered and named based on their abilities to stimulate follicle-stimulating hormone (FSH) secretion from cultured pituitary gland. According to current dogma, activin B produced by pituitary gonadotrope cells stimulates transcription of the FSHβ subunit gene (Fshb). Challenging this idea, Inhbb knockout mice, which cannot make activin B, exhibit elevated serum FSH levels. In addition, gonadotrope-specific deletion of the canonical activin type I receptor, Acvr1b, does not impair FSH synthesis or fertility in vivo. Nonetheless, mice lacking the activin type II receptor (Acvr2) exhibit FSH deficiency. We therefore hypothesized that a TGFβ protein, other than activin, is the primary intra-pituitary regulator of FSH synthesis. According to RNA-seq analysis, growth differentiation factor 11 (Gdf11) is highly expressed in purified murine gonadotropes. In LβT2 cells, a gonadotrope cell line, ACVR2 knockdown impaired Fshb promoter activity induced by activin A and GDF11 in a similar manner. In primary pituitary cultures, activin A and B signaled via ACVR1B to induce Fshb transcription, whereas, GDF11 signaled through both ACVR1B and a second type I receptor, TGFBR1. In vivo, gonadotrope specific-deletion of Tgfbr1 resulted in decreased litter size in female conditional knockouts compared to controls. The data suggest that an endogenous ligand that signals via TGFBR1 in gonadotropes is required for normal fertility. We are currently generating gonadotrope-specific Gdf11 knockout mice to definitively determine whether or not GDF11 is the relevant TGFβ ligand regulating FSH in vivo. The results of these analyses will redefine our understanding of mechanisms of FSH synthesis.

Funding Sources: Supported by CIHR MOP-133394.
IN VITRO EFFECTS OF A MIXTURE OF GENISTEIN AND THE PLASTICIZER MEHP ON TESTICULAR CELLS

Annie Boisvert¹,³, Steven Jones¹,², Andrada Naghi⁴, Francoise Hullin-Matsuda⁵, Peter Greimel⁵, Toshihide Kobayashi⁵, Vassilios Papadopoulos¹,⁴ and Martine Culty¹,²,³,⁴

¹The Research Institute of the McGill University Health Centre, ²division of Experimental Medicine, Departments of Medicine³ and Pharmacology & Therapeutics⁴, McGill University, Montreal, Quebec, Canada. ⁵Lipid Biology Laboratory, RIKEN Institute, Wakoshi, Saitama, Japan

We previously reported the ability of a mixture of two common environmental chemicals, the phytoestrogen genistein (GEN) and the phthalate plasticizer DEHP, to induce different short and long term effects in the testes of rats exposed in utero to the mixture compared to control rats. Some of the effects observed were not replicated by exposure to the single compounds, stressing the importance of assessing mixtures in toxicological studies. These studies identified neonatal and adult Leydig and germ cells as targets of GEN+DEHP. To further investigate the direct effects and potential mechanisms involved in GEN and DEHP effects, we treated mouse MA-10 tumor Leydig cells and isolated primary rat neonatal germ cells (gonocytes) and enriched Leydig cells with varying concentrations of GEN and MEHP, the principle bioactive metabolite of DEHP. A panel of genes relevant for Leydig cell function, germ cell and oxidative stress markers were analyzed, as well as lipid homeostasis in MA-10 cells and gonocytes.

Combined GEN + MEHP at 10 μM, a concentration similar to levels found in human blood, exerted different effects on lipids in MA-10 cells and gonocytes, increasing the levels of several neutral lipid and phospholipid classes in MA-10 cells, but decreasing them in gonocytes. The mixture at 10 μM also stimulated basal MA-10 progesterone production by 2-fold. In agreement with these lipid alterations, the transcripts of several cholesterol, steroidogenesis and phospholipid mediators were increased in MA-10 cells, uniquely by the mixture. In gonocytes, the 10 μM GEN + MEHP mixture altered the expression of several germ cell markers. The mixture increased the expression of antioxidant genes both in gonocytes and enriched primary Leydig cells. Interestingly, genistein alone affected Leydig cells antioxidant responses, differently from the results previously obtained in whole testes.

These results suggest that the combination of GEN + MEHP deregulates testicular cell function. Taken together with our previous in vivo studies, these data indicate different sensitivity of Leydig and early germ cells to genistein and DEHP/MEHP, and further suggest that the early oxidative stress responses observed in vivo involve various testicular cell types.
EFFECT OF PRENATAL EXPOSURE TO 1,2-CYCLOHEXANE DICARBOXYLIC ACID DIISONONYL ESTER (DINCH) ON OFFSPRING TESTICULAR FUNCTION

Enrico Campioli\textsuperscript{1,2}, Matthew Lau\textsuperscript{1,4}, Sunghoon Lee\textsuperscript{1,3} and Vassilios Papadopoulos\textsuperscript{1,2,3,4}

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1,2-Cyclohexane dicarboxylic acid diisononyl ester (DINCH) is a plasticizer introduced in 2002 in the European market for use in plastic materials and articles intended to come into contact with food. Phthalate plasticizers, have been associated with the development of endocrine and reproductive diseases. Recent studies in our laboratory showed that one of the metabolites of DINCH, 1,2-dicarboxylic acid mono isononyl ester (MINCH), was able to promote in vitro rat preadipocyte differentiation through the peroxisome proliferator-activated receptor (PPAR)-\(\alpha\) pathway in a manner similar to the phthalate metabolite MEHP. The purpose of the present work was to determine whether in utero exposure to DINCH could affect testis function in adult male Sprague-Dawley rats exposed in utero to 1, 10 and 100 mg DINCH/kg/day from gestational day 14 until birth.

No effect on body weight and anogenital distance was observed at PND 3, 6 and 60. Gene markers of somatic and germ cell function in the testis were increased at PND 3 and 6, suggesting a potential perturbation of neonatal testis function in response to DINCH. In contrast, in PND 60 animals, opposite effects were observed; e.g. Ar levels were significantly reduced starting at 10 mg DINCH/kg/day. Moreover, DINCH exposure resulted in a dose-dependent reduction of serum testosterone levels and seminal vesicle weight. Taken together, these results suggest that exposure to DINCH during a fetal period critical for testicular development induces changes in the endocrine and reproductive system of the male rat offspring.

Funding Sources: Supported by CIHR grant MOP-111131 and CRC
POSTER 9

MODELLING TRANSIENT KDM1A-LOSS ON THE EMBRYONIC EPIGENETIC LANDSCAPE

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2Département de Biochimie, Faculté de Médecine et Sciences de la Santé, Université de Sherbrooke, Sherbrooke, Québec, Canada
3Département Obstétrique-Gynécologie, Faculté de Médecine, Université de Montréal, Québec, Canada

During prenatal life, adverse in utero conditions via environmental factors are thought to perturb epigenetic marks in the embryonic program that could potentially enhance the risk of adverse health consequences following birth. To date, we have little information if a temporary deficiency in histone modifying enzymes in early development could initiate inherited epigenetic dysregulation on histone residues and compromise future genes regulations. Here we propose to induce perturbations onto the epigenetic program of embryonic stem cells (ES) by targeting Kdm1a (lysine specific demethylase-1a) to identify which epigenetic modifications and interactions associated with developmental processes are susceptible to inherited dysregulation, i.e. the cell-to-cell transmission of epigenetic errors. Kdm1a is fundamental for both embryo and brain development. We will use a tetracycline-controlled transcriptional (tet-off) system to induce a transient Kdm1a, a regulator targeting H3K9me1/2 and H3K4me1/2, in embryonic stem cells. We postulate that the transient loss of Kdm1a will remodel epigenetic interactions in the embryonic epigenome and introduce inherited dysregulation. My goal is to generate an ES cell line with malleable Kdm1a expression, to be used in the future to determine the impact of transient Kdm1a expression on the embryonic epigenetic landscape. These experiments will allow us to identify which epigenetic marks and interactions associated with developmental processes are the most vulnerable to dysregulation of Kdm1a activity during early life.

Funding Sources: Supported by CHUSJ Research Center
POSTER 10

EARLY EMBRYONIC EPIGENETIC PERTURBATIONS AND FETAL ALCOHOL SPECTRUM DISORDERS.

Lisa-Marie Legault1,2, Virginie Bertrand-Lehouillier1,2, Roxane Landry1, Christine Kirady1, Maxime Caron1, Perrine Gaub1, Daniel Sinnett1,2,3, Serge McGraw1,2,4

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Recent studies show that prenatal alcohol exposure (PAE) can trigger altered epigenetic modifications in brain cells. These alterations are said to be implicated in the development of Fetal Alcohol Spectrum Disorders (FASD). However, the consequences of a PAE during the first few days of embryonic life on the future epigenetic landscape of the brain remain unknown. Our research hypothesis is that a PAE during pre-implantation will initiate specific and permanent DNA methylation dysregulation. Consequently, we believe that these epigenetic alterations will be perpetuated and amplified in the developing brain, leading to abnormal brain functions associated to FASD.

To test this, we instigated FASD in 8-cell embryos by injecting ethanol (2x 2.5g/kg) to pregnant mouse at 2.5 days of pregnancy (E2.5). We collected FASD (ethanol) and control (saline) embryos (E10.5), isolated the forebrain and performed Reduced Representation Bisulfite Sequencing (RRBS) to establish genome-wide quantitative DNA methylation profiles. A total of 830 differentially methylated regions, mostly hypermethylated, were affected in FASD embryos. We also observe heterogeneity between FASD samples, reflecting the variety of symptoms and phenotypes present in FASD children. A gene ontology analysis linked these perturbations with processes implicated in FASD abnormalities. Our study establishes that PAE in the first days of pregnancy can cause epigenetic dysregulations that leads to permanent damages to brain cells. Our results allow us to have a better understanding of how epigenetic perturbations can alter the normal function of the brain and lead to neurodevelopmental disorders present in children with FASD.
SPECIFIC ALTERATIONS IN THE HISTONE MODIFICATION LANDSCAPE AS A CONSEQUENCE OF TRANSIENT DNMT1 DEFICIENCY IN MOUSE ES CELLS

Virginie Bertrand-Lehouillier¹, Lisa-Marie Legault¹, Roxane Landry¹, Christine Kirady¹, Maxime Caron¹, Perrine Gaub¹, Nicolas Gévry³, Rich Chaillet², Daniel Sinnett¹, Serge McGraw¹.

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Genome-wide demethylation and remethylation of DNA during early embryogenesis is essential for mammalian development and genome integrity. Through mostly unknown mechanisms, imprinted germline differentially methylated domains (gDMDs) are able to retain their methylation profiles during the reprogramming period. Using an embryonic stem (ES) cell line, in which Dnmt1 is regulated by a tet-off system (Dnmt1tet/tet ES cells), we recently showed that a temporary lack of DNMT1 activity triggers the inherited loss of gDMDs and gDMD-like DNA methylation profiles, whereas most regions of the genome are able to recover original DNA methylation levels. Here, using the same Dnmt1tet/tet ES cell system, we investigate if a transient lack of DNMT1 activity prompts rearrangements in the histone mark landscape. Histone modification (H3K4me3, H3K27me3, H3K27ac) profiles were assessed using chromatin immunoprecipitation-sequencing (ChIP-Seq) and crossed-analyzed with DNA methylation profiles generated by reduced representation bisulfite sequencing (RRBS). We found that perturbation in histone modifications following temporary lack of DNA methylation maintenance is not necessarily associated with neighboring inherited loss of DNA methylation. However, in other regions, we believe that alteration in some histone marks could impede the recruitment of DNMTs; suggesting that alterations in the histone modification landscape may prevent the proper recruitment of DNMT1. The present study presents new perspectives on how alterations in normal DNA methylation and histone modification cross-talk could explain epigenetic inherited dysregulation events during embryo development.
GLOBAL DNA METHYLATION IN HERRING GULL COLONIES FROM HIGHLY CONTAMINATED AND UNCONTAMINATED SITES

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Early life stages are extremely sensitive to exposure to environmental contaminants. This is also the period when epigenetic marks such as DNA methylation are established. Recent research suggests that disruptions to the epigenome early in life can cause persistent, lifelong consequences in individuals.

The goal of this project was to evaluate global DNA methylation as a biomarker for embryonic exposure to environmental contaminants in herring gull (Larus argentatus) in the Great Lakes. Research has shown that DNA methylation is affected by many classes of contaminants, (e.g. metals, organic contaminants, pesticides), in many animal models. We hypothesized that herring gulls living and feeding in highly contaminated sites would exhibit DNA hypomethylation compared with individuals from control sites. Fertile unincubated herring gull eggs were collected from 3 different colonies in Lake Ontario. Previous research indicates that eggs from these colonies exhibit a gradient of contaminant levels (Hamilton Harbour >Salmon Island >Cornwall). Eggs were artificially incubated for ~22 days, and blood, brain and liver tissues were collected from the embryos. The remaining egg contents were preserved for chemical analysis. Global DNA methylation was assessed in each tissue type via LUminometric Methylation Analysis (LUMA). Levels of global DNA methylation were extremely stable across individuals, and there was no significant difference among the 3 herring gull colonies. Significant differences were detected in DNA methylation levels across tissue type, and these were consistent among colonies (blood>brain>liver). Future analyses will focus on the levels of contaminants in the eggs and methylation specific genes within the samples that were collected.

Funding Source: Supported by NSERC.
PAHS CAUSE TRANSIENT CHANGES IN CYP1A EXPRESSION AND PERSISTANT CHANGES IN DNA METHYLATION IN THE DEVELOPING CHICKEN EMBRYO

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Polycyclic aromatic hydrocarbons (PAHs) are environmental pollutants, which can cause embryomortality and sublethal developmental effects in birds. The response to PAHs is partly mediated by the aryl hydrocarbon receptor (AHR). Activation of the AHR leads to increased expression of several genes, (e.g. CYP1A). In addition, exposure to some AHR ligands may result in changes in DNA methylation; a biochemical process that can lead to changes in gene expression. The goal for this project was to determine if early life exposure of birds to PAHs affects methylation of AHR responsive genes. This is important because changes in DNA methylation early in life may affect how an individual responds throughout life.

To test our hypothesis, we exposed chicken embryos to the PAH Benzo[k]fluoranthene (BkF). Liver samples were collected at several stages of development and analyzed for CYP1A gene expression and promoter methylation status. We measured a significant increase (27-fold) of gene expression at embryonic day 10 (ED10). At ED19, expression levels were back to baseline levels. This is not surprising, as most of the BkF is expected to be metabolized by the embryo at this stage. However, we also detected a small but significant increase in DNA methylation at the CYP1A promoter that persisted after hatching. These findings suggest that transient changes in gene expression may be accompanied by more persistent alterations to DNA methylation. Ongoing studies are focussed on whether this change in DNA methylation modifies the induction of CYP1A isoforms upon re-exposure to PAHs.

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POSTER 14

HUWE1 FUNCTIONS IN ESTABLISHMENT OF SPERMATOGONIA BY MODULATING THE DNA DAMAGE RESPONSE THROUGH REGULATION OF H2AX EXPRESSION

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An increasing number of studies have demonstrated the importance of ubiquitination in spermatogenesis and fertilization. We previously discovered a 480 kDa ubiquitin ligase named Huwe1 from testis and identified its ubiquitination activity towards all 4 core histones in vitro. Through germ cell inactivation of Huwe1 in mice, we demonstrated that Huwe1 is required for the establishment and maintenance of spermatogonial stem cells (SSC). Loss of Huwe1 resulted in infertility, degeneration of the germ cells and was associated with an increased number of γH2AX intensively staining foci. H2AX is a histone variant that is phosphorylated to become γH2AX and recruited to sites of double strand breaks (DSBs). This suggested that loss of Huwe1 resulted in a hyperactivation of the DNA damage response (DDR). To explore the molecular mechanism, we established a cell culture model in which Huwe1 was depleted in the C18-4 type A spermatogonia cell line. Loss of Huwe1 resulted in a 30% decrease in proliferation rate which mimicked the germ cell depletion seen in vivo. The cell number decrease was associated with a 50% increase of H2AX and γH2AX. Clonogenic assay revealed a 60% decrease in colony formation in Huwe1 depleted cells and this was further exacerbated when cells were exposed to an inducer of DSBs. Immunoprecipitation revealed an interaction between Huwe1 and H2AX, suggesting that H2AX is a substrate of Huwe1. Exposure of cells to DNA damage induced by X-ray irradiation or the radiomimetic damaging agent hydroxyurea, resulted in a prolonged induction of γH2AX. To determine whether the increased H2AX expression is responsible for the abnormal DDR and cell death, we tested the effects of silencing H2AX in Huwe1 depleted cells. Decreasing H2AX levels in these cells to basal levels reversed the defect in cell proliferation. Thus, Huwe1 likely influences cell growth by controlling the DDR through ubiquitination and degradation of H2AX.
ROLE OF FOLIC ACID SUPPLEMENTATION IN THE PREVENTION OF EPIGENETIC AND BIRTH DEFECTS ASSOCIATED WITH THE USE OF ASSISTED REPRODUCTION

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Infertility affects approximately 10% of couples and is an important factor contributing to the relatively high proportion of children being conceived using assisted reproductive technologies (ART). Adverse outcomes such as decreased birth weight, an increased incidence of birth defects and preeclampsia have been associated with the use of ART. These procedures have been shown to alter epigenetic (DNA methylation) programming events during germ cell and early embryo development. For proper establishment of DNA methylation profiles during these stages of development, the availability of methyl donors such as dietary folate is essential. The objective of my research is to determine whether folic acid supplementation can prevent birth defects and epigenetic abnormalities associated with ART. Female mice (n=8-10/ART or control group) are exposed to control (2 mg folic acid/kg diet), low dose (8 mg folic acid/kg diet) and high dose (20 mg folic acid/kg diet) folic acid-supplemented diets six weeks before in vitro fertilization or natural mating; diets are continued throughout gestation. To date, mouse ART consists of treatments used in human ART; a combination of superovulation, in vitro fertilization, embryo culture to blastocyst and embryo transfer has been set up and optimized. Embryos and placentas collected at mid-gestation will be examined for abnormalities and used for the assessment of imprinted gene methylation (bisulfite pyrosequencing) and expression (Q-RT-PCR). Genome-wide DNA methylation profiling studies will also be conducted. This study will help determine whether folic acid supplementation in the ranges used clinically is beneficial or deleterious in pregnancies resulting from ART.

Funding Sources: Supported by CIHR.
The phenomenon of epigenetic inheritance has been observed in models ranging from worms to mice, however the underlying molecular mechanisms remain unknown. Recently we showed that disruption of histone methylation in developing sperm leads to reduced survivability and abnormal development of offspring (Siklenka et al., 2015). Sperm chromatin is unique due to the high degree of chromatin compaction achieved after replacement of most histones by protamines. Between 1% and 15% of histones are retained in sperm of mice and men, respectively. Initial studies of the sperm epigenome determined that nucleosomes were preferentially enriched at gene regulatory regions (Brykczynska et al., 2010; Hammoud et al., 2009). In contrast, using different approaches, Carone et al., 2014 and Samans et al., 2014 observed genome-wide nucleosome retention and reduced representation at gene-regulatory CpG dense regions.

Our objective was to use ChIP-seq, in combination with pooled samples from men (n=30) and mice (n=3X3) to generate high coverage, robust data sets for determination of H3K4me3 localization in the sperm genome. Methods: We prepared native chromatin followed by immunoprecipitation by H3K4me3, and sequencing. Comparative analysis was performed testing various parameters in Homer (v4.8), MACS, MACS2, and R/Bioconductor. Results: DNA was sequenced at a depth of over 160 million reads in sperm from men and at a depth of 30 million reads in sperm from mice. Our analysis confirms that H3K4me3 is enriched at TSS. Intriguingly, although nucleosomes were not enriched at intergenic sequences we observed a sizable portion of positive H3K4me3 peaks that may associate with distal gene regulatory regions.

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EPIGENETIC PROGRAMMING ESTABLISHED IN SPERMATOGONIA IS MAINTAINED THROUGH SPERMATOGENESIS

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The environmental exposures and lifestyle of parents can alter the development and phenotypes of offspring. This heritable environmental information is transmitted by gametes and involves epigenetic mechanisms including histones, DNA methylation and non-coding RNA. We recently determined that disruption of histone H3 dimethylation at the lysine 4 (H3K4me2) in spermatogenesis has transgenerational consequences in the health and development of offspring (Siklenka et al., Science, 2015). However, little is known about when in spermatogenesis histone methylation is established. We hypothesize that epigenetic modifications to histones present in spermatogonia persist through spermatogenesis and can be transmitted to offspring via the sperm. Our objective was to determine what genomic regions bearing H3K4me2 in an enriched population of Spermatogonial Stem Cells (SSC) and progenitors retain this mark in sperm. Methods: We isolated a mouse spermatogonia population enriched in SSC/progenitors and performed ChIP-sequencing for H3K4me2. Computational approaches were used to compare H3K4me2 in this spermatogonia population to sperm. Results: Our analysis revealed that H3K4me2, in the SSC/progenitors population, is enriched at the Transcription Start Site region (TSS region: -1kb to +1kb around the TSS) of more than 12,000 genes. H3K4me2 also localizes at intergenic and distal gene body (> +3kb) regions including at testis specific enhancers (Mouse ENCODE, Shen et al., Nature, 2012). Despite the retention of only 1% of histones in mouse sperm, 40% of the H3K4me2 peaks present in spermatogonia are conserved in sperm. These findings suggest that if epimutations are induced in spermatogonia they may influence the health and development of offspring.

Funded by the Canadian Institutes of Health Research (CIHR)
Preimplantation mammalian development consists of a series of morphological and molecular changes leading to the formation of a blastocyst, which is ready to implant into a mother’s womb. After fertilization, an 8-cell stage embryo initiates the first aspect of divergent cell lineage specification, resulting in the generation of the trophectoderm (TE) and the inner cell mass (ICM). Cell polarity, particularly the presence or absence of an apical domain in a cell plays an essential role for TE/ICM specification. 8-cell blastomeres initiate formation of the apical domain and the subsequent asymmetric division controls whether daughter cells inherit the apical domain to generate the first distinct cell populations, polar outer and apolar inner cells. Lineage specific gene expression is promoted in both outer polar and inner apolar cells to adopt the TE and ICM fates, respectively. The hippo signalling pathway is a key signalling component to establish lineage specific transcription. The main transcriptional activator is known as YAP, forms a transcriptional complex with TEAD4, within the outer cells to induce TE specific genes. Conversely, YAP can also be regulated through phosphorylation, inhibiting its entry to the nucleus and thus preventing the TE gene expression in the ICM. An adapter protein, AMOT, is proposed to be a key regulator of Hippo signalling activation. However, our analysis suggests involvement of another molecule in the early stage of lineage specification. Currently it is unknown what molecules regulate the formation of the apical domain and differential Hippo activation.

Funding Source: Center for Research in Reproduction and Development (CRRD)
DEVELOPMENTAL COMPETENCE OF HOLSTEIN CALF OOCYTES

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Recent advances in *in vitro* embryo production technologies may allow for the production of offspring from prepubertal animals. However, past literature has reported high oocyte yields recovered from calves aged 2-6 months old but low embryo development rates following *in vitro* fertilization and culture. The objective of this study is to characterise the developmental competence of oocytes from young calves. We report herein the oocyte and embryo yields obtained from 6 Holstein calves that were subjected to gonadotropin stimulation and laparoscopic ovum pick-up (LOPU) every 2 weeks, starting at 2 and ending at 5 months of age. A total of 766 follicles were aspirated (avg. 17/calf/session) resulting in 625 cumulus-oocyte complexes (COCs) recovered (avg. 14/calf/session; 82% recovery rate). A total of 457 (73%) COCs were graded eligible for IVM, of which 353 cleaved (77%) and 109 (24%) reached a viable blastocyst stage at the end of IVC. It was found that of the embryos that cleaved but failed to reach the blastocysts stage, approximately 50% arrested their development prior to 8-cell stage, the main period of embryo genome activation in bovine. The incidence of polyspermy was also found to be higher than normally reported after IVF in adult cows. Nevertheless, approximately two viable blastocysts/calf/session were produced. Moreover, blastocyst transfer to surrogate cows produced pregnancy and calving rates comparable to embryos from adult cows. At the end of the experiment, no adverse health or fertility effects were observed in the animals. This indicates that LOPU/IVP can be applied in Holstein calves to reduce the inter-generation interval and produce more offspring from elite animals.

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REPAIR OF DNA DOUBLE-STRAND BREAKS IN EARLY DEVELOPING EMBRYOS IS MAINLY ACTIVATED BY THE HOMOLOGOUS RECOMBINATION PATHWAY

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DNA double-strand breaks (DSBs) are known to affect early embryo development. The homologous recombination (HR) and the nonhomologous end-joining (NHEJ) are the main pathways activated in response to DSBs to promote DNA repair in somatic cells. However, the importance of each pathway for DSBs repair in early embryo development has not been determined. Therefore, the objective in this study was to investigate the importance of the HR and NHEJ pathways in DNA repair and embryo development to the blastocyst stage. In the first experiment, embryos were cultured in the presence of specific chemical inhibitors of HR, NHEJ or both pathways until the blastocyst stage. Inhibition of HR or both pathways, but not NHEJ, increased DNA damage, reduced blastocyst formation and increased the proportion of apoptotic cells. To further confirm these findings, dicer-substrate siRNA (DsiRNA) were microinjected into oocytes to inhibit the activation of HR (ATM and ATR DsiRNAs), NHEJ (DNA-PK DsiRNAs) or both (ATM, ATR and DNA-PK DsiRNAs) pathways. The knockdown of HR or both pathways reduced development to the blastocyst stage and embryo quality. Moreover, knockdown of ATM, but not ATR, increased DNA damage in day 5 embryos and consequently reduced blastocyst formation and quality. Also, ATM knockdown increased (P<0.05) mRNA levels for genes involved in DNA repair. Taking together, these findings demonstrate that the HR is the main pathway responsible for DSBs repair in early developing embryos. Our findings also indicate that the HR pathway is mainly activated by ATM during early embryo development.

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POSTER 21

XB1 Dysregulation by CRISPR/Cas9-Mediated Gene Editing During Porcine Embryo Early Development

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Early developing embryos are very sensitive to their developmental milieu. For instance, variations in temperature, pH or culture media composition can trigger endoplasmic reticulum (ER) stress. ER stress has been shown to reduce early embryo development and embryo quality. In response to ER stress, embryos may activate coping mechanisms, such as the unfolded protein response (UPR), to re-establish ER homeostasis. The X box binding protein (XB1) is one of the main transducers of the UPR. Under ER stress, XB1 mRNA is unconventionally spliced by IRE1α to yield its activated isoform (XB1s), which allows expression of genes involved in protein folding, transport and degradation. XB1s has been detected in oocytes and early stage embryos of different species, including Drosophila, Xenopus, zebrafish, mice and pigs, suggesting an important role during early embryo development. In this study, we used the CRISPR/Cas9 gene editing technology to evaluate the effect of XB1 dysregulation during development of porcine embryos in vitro. Pig zygotes were produced by intracytoplasmic sperm injection into in vitro-matured oocytes. Cas9 mRNA was injected alone (control) or with one or two single guide RNAs (sgRNA) targeting XB1 gene sequences. We found that embryo cleavage was similar between the control and treated groups, but development to the blastocyst stage was substantially reduced in embryos injected with either of two or combined XB1 gene-targeted sgRNAs. The findings suggest that XB1 activity is required for maintenance of ER homeostasis after genome activation in developing porcine embryos.

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EX VIVO ANALYSIS OF CHORIOALLANTOIC FUSION SHOWS THAT TMED2 IS REQUIRED IN THE CHORION FOR PLACENTAL DEVELOPMENT

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Development of the mouse placenta proceeds rapidly after the allantois and chorion attach through a process called chorioallantoic attachment. As a consequence of chorioallantoic attachment, a subset of chorionic cells in proximity of the allantois differentiate into syncytiotrophoblast cells and form invaginations into which allantoic-derived embryonic blood vessels migrate to form the labyrinth layer. Chorioallantoic attachment and subsequent differentiation events required for placental development have remained poorly investigated partly due to being inaccessible to ex vivo analysis. Here, we report conditions for ex vivo culture of pre-attachment chorion and allantois. Under these culture conditions, explants of pre-attachment allantois and chorion attached and showed extensive mixing of chorionic and allantoic cells. We confirmed that the allantois was required for expression of the syncytiotrophoblast cell marker Gcm1. In addition, we found that maintained expression of the spongiotrophoblast cell marker Tpbpa also depended on chorioallantoic attachment. We tested the efficacy of this ex vivo model by examining the tissue-specific requirement for Tmed2, a member of the transmembrane emp24 domain (TMED) protein family, required for normal placental development. Recombinant cultures of Tmed2 null and wild type chorion/allantois revealed a role for Tmed2 in cell survival and for mixing of chorionic and allantoic cells. Thus we report the first successful ex vivo model of pre-placental tissues before chorioallantoic attachment, and show that this explant system can be used to reveal tissue-specific requirements of genes required for placental development.
THE BINDER OF SPERM HOMOLOG 2 (BSPH2) GENE KNOCKOUT BY CRISPR/CAS9 SYSTEM AND THE EFFECT ON IN VIVO FERTILITY

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\textbf{Introduction:} Sperm capacitation is crucial for the encounter and fusion of gametes. During capacitation important changes on the sperm membrane are mediated by a family of proteins, ubiquitous among mammals, termed Binder of SPerm (BSP). These BSP proteins are expressed exclusively in the male reproductive tract where they bind choline phospholipids on sperm membranes and promote capacitation. Our recent studies have shown that one BSP family member BSPH1 is expressed in human epididymes whereas two are expressed in murine epididymes, BSPH1 and BSPH2.

\textbf{Objectives:} elucidate the functions of BSP proteins in fertilization \textit{in vivo}.

\textbf{Method:} Single (either \textit{Bsph1\textsuperscript{-/-}} or \textit{Bsph2\textsuperscript{-/-}}) and double (\textit{Bsph1\textsuperscript{-/-}} and \textit{Bsph2\textsuperscript{-/-}}) knockout mice were constructed using the revolutionary new targeted genome editing technique of Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated nuclease 9 (CRISPR/Cas9).

\textbf{Results:} The results from \textit{Bsph2\textsuperscript{-/-}} mice are presented here. Progeny carrying mutated \textit{Bsph2} alleles were screened by PCR as well as qPCR, and the deficit in BSPH2 mRNA expression was confirmed by RT-PCR. The preliminary data suggest that male \textit{Bsph2\textsuperscript{-/-}} mice are fertile in terms of average pup weight, average litter size, and number of delivery. Further studies will be carried out to evaluate sperm functions (motility, viability, capacitation, acrosome reaction), and reproductive organ morphology. We also plan to characterize \textit{Bsph1\textsuperscript{-/-}} mice as above.

\textbf{Conclusion:} Moreover, since \textit{Bsph1} and \textit{Bsph2} may exert overlapping function(s), it will be important to characterize the double knockout as well.

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POSTER 24

COMPARING THE EXPRESSION PROFILE OF GENES INVOLVED IN FOLLICULAR GROWTH AND OOCYTE MATURATION IN GRANULOSA CELLS OF PREPUBERTAL AND MATURE HOLSTEIN COWS

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Canadian dairy genetics are renowned worldwide and advanced reproductive technologies play an important role in accelerating genetic improvement. As a result, there is a growing interest in producing offspring from genetically superior females at prepubertal ages. However, previous reports suggest lower in vitro embryo development rates are obtained from calf oocytes. To explore potential causes associated with the lower developmental competence of prepubertal bovine oocytes, we compared mRNA abundance of candidate genes in mural granulosa cells (GCs) of prepubertal and mature animals. Bi-monthly laparoscopic ovum pick-ups (LOPUs) were performed on six gonadotropin-stimulated Holstein heifers from 2 to 6 months of age. Cumulus oocyte complexes (COCs) and GCs were collected from 2-10mm follicles and the oocytes were matured, fertilized and cultured in vitro using standard industry procedures for adult cow oocytes. The prepubertal GC samples were subdivided into three animal-age groups, i.e. 2-3, 4-5, and 6 months old and compared to GCs collected from eight gonadotropin-stimulated adult (17-22 months old) Holstein cows. The average size of follicles aspirated from the prepubertal animals was between 5-6mm compared to 7-10mm in the mature animals. For the candidate genes evaluated, we observed no significant difference in mRNA levels of FSHr, STAR, CYP19A1, CX43, ATP2A2 and XIAP between prepubertal and adult samples. However, LHr mRNA was only detected in samples from mature cows, and CALM and FOXO1 mRNA levels were significantly higher in GCs of prepubertal compared to mature cows (Student T-test, P<0.05). Additional analyses are being conducted to evaluate the expression of other candidate genes.

Funding Sources: Supported by NSERC, and in collaboration with Boviteq
Infertility caused by the loss of spermatogonial stem cells (SSC) is a common consequence of chemo- and radiation therapy in cancer survivor patients. Currently the only effective approach to preserve fertility in these patients is sperm banking, which has a low success rate (25%) and is not a viable option for prepubertal boys. The alternative treatment is to harvest and cryopreserve the SSC before therapy and then inject the SSC into the testes later; SSCs migrate through the blood testes barrier (BTB) to the stem cell niche to regenerate spermatogenesis. To improve the efficiency of this technique we are transiently opening the Sertoli cells’ tight junctions (TJ) by targeting the claudin family of TJ proteins. Claudins contain two extracellular loops (EL); the first (EL1) helps to determine size and ion selectivity of the barrier and the second (EL2) interacts with claudins in the apposing cell. The non-toxic C-terminal domain of the enterotoxin of Clostridium perfringens (C-CPE) binds to the EL2 of a subset of claudins, removing them from the TJ and opening the barrier. Some nutraceuticals have shown to affect the claudin expression modifying the barrier properties. Efforts are underway to show how C-CPE and nutraceuticals are able to modify TJ for effective but transient BTB weakening or breakdown.

Funding sources: Supported by CIHR
Spermatogenesis involves crucial, highly regulated transitions between developmental programs such as mitotic proliferation, meiosis and differentiation. The ubiquitin proteasome system plays a significant role in protein turnover and cellular remodeling and may be involved in these transitions. We previously identified ubiquitin ligase Huwe1 in the testis and showed that inactivating it in gonocytes results in a delay in their mitotic re-entry and leads to spermatogonial depletion. Here we inactivated it in differentiating spermatogonia by expressing Cre recombinase using the Stra8 promoter. Huwe1⁺/⁻ males (KO) were infertile. The average testes weight of adult KO was only 30% of the WT. Morphological analysis of adult testis revealed that the KO tubules had fewer spermatocytes and spermatids. To identify the specific stage(s) at which the spermatogenic defect was occurring, the tubules were synchronized using WIN 18,446/retinoic acid. With this approach, when sacrificed at a time point after completion of the first wave of spermatogenesis, a down-regulation of markers of spermatogonial differentiation (Stra8, Dazl, Sohlh1, Sohlh2) was observed. In the first wave, there were 84% fewer pre-leptotene spermatocytes in the KO along with a 44% decrease in BrdU incorporation indicating a defect in meiotic entry. The KO testes showed a down regulation in early meiotic markers (Spo11, Smc1b, Sycp1, Sycp3). Chromosome spread analysis using SCP3 (marker of meiotic progression) revealed severe degeneration of spermatocytes (5.6% WT vs. 56.8% KO) with the percentage of pachytene spermatocytes falling by 96% in the KO. Currently, studies are being undertaken to understand Huwe1’s role in meiotic progression better.

Funding source: FRSQ, Genome Quebec, CIHR-China
POSTER 27

SFRP4 FUNCTIONS AS A PHYSIOLOGIC INHIBITOR OF GONADOTROPIN SIGNALING IN MOUSE GRANULOSA CELLS

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WNTs are signaling molecules that regulate a variety of ovarian processes, including granulosa cell (GC) proliferation and differentiation, steroidogenesis and ovulation. Secreted Frizzled Related Proteins (SFRPs) are known antagonists of WNT signaling, but their role in the ovary is poorly understood. The expression of \textit{Sfrp4} is strongly induced in rodent GCs in response to gonadotropins, but for what purpose remains unknown. We have generated Sfrp4\textsuperscript{--/} mice, which presented increased rates of ovulation and were hyperfertile. To determine the cellular and molecular basis for this phenotype, microarray analyses of SFRP4-treated cultured GCs were performed, which revealed that SFRP4 treatment results in changes in expression levels of \textgreater 3000 genes. Most interestingly, SFRP4 markedly suppressed the expression of important FSH-responsive genes, and corresponding increases in the expression of these genes were found in the GCs of Sfrp4\textsuperscript{ KO} mice. GCs isolated from Sfrp4\textsuperscript{--/} mice and cultured in the presence of FSH or LH were hyper-responsive to gonadotropins relative to GCs from wild-type mice. Likewise, pre-treatment of cultured GCs with SFRP4 suppressed the effects of both FSH and LH on their target genes, and this suppression was found to be glycogen synthase kinase 3β (GSK3β)-dependent. cAMP responsive element binding (CREB) protein levels were found to be increased in GCs of Sfrp4\textsuperscript{ KO} mice, and decreased in GCs cultured in the presence of SFRP4. Taken together, our data suggest that SFRP4 functions as a negative regulator of female fertility, and suppresses gonadotropin action by a mechanism involving both GSK3β and CREB.

Funding Sources: Supported by RQR and CIHR.
HGSNAT (HEPARIN-A-GLUCOSAMINIDE N-ACETYLTRANSFERASE) GENE INACTIVATION AFFECTS THE LYSOSOMAL INTEGRITY OF EPIDIDYMAL EPITHELIAL CELLS AND THE MORPHOLOGY OF SPERMATOZOA IN ADULT MICE. AN ELECTRON MICROSCOPE STUDY

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The epithelial cells lining the epididymis provide a unique environment for sperm maturation controlled in part, by the endocytosis of substances from the lumen. Heparan sulfate (HS) is a component of basement membranes and the apical surface of cells. In many cell types, HS is degraded after endocytosis in a stepwise fashion in lysosomes by the action of several enzymes, including HGSNAT. In mice, inactivation of the HgsNat gene shows defects in sperm motility and in vitro fertilization. The objectives of this investigation were to determine the morphological effects of HgsNat inactivation on the epithelial cells of the epididymis and epididymal spermatozoa of adult mice by electron microscopy (EM). In WT mice, the epithelial principal cells contained several small to medium sized dense spherical lysosomes. This contrasted the HgsNat deficient mice, where numerous large to gigantic sized looking vacuoles of different shapes appeared both supranuclearly and infranuclearly; they were confirmed to be lysosomes by immunohistochemical analysis. Such vacuoles were also prominent in basal, halo and myoid cells, all of which were greatly increased in size. Occasional huge cells occupied the mid/basal region of the epithelium which based on morphological criteria were identified as detached clear cells. In the lumen, numerous spermatozoa presented abnormal tails and head shapes. All of the above observations, although evident in all epididymal regions, were especially prominent in the corpus region. Our results reveal that upon Hgsnat inactivation, defects occur to sperm tail formation in the testis, as well as a severe alteration of epididymal epithelial cell morphology which may affect sperm maturation.

Supported by NSERC and CIHR.
ABNORMALITIES OF HEPARIN-ALPHA-GLUCOSAMINIDE N-ACETYLTRANSFERASE (HGSNAT) GENE INACTIVATION AS SEEN IN THE TESTIS AND EPIDIDYMIS OF ADULT MICE. AN IMMUNOHISTOCHEMICAL AND MORPHOLOGICAL ANALYSIS

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Heparan sulfate is a component of extracellular matrix, basement membranes and apical cell surface, and is abundant in testis and epididymis. Heparan sulfate is degraded in a stepwise fashion by the action of three glycosidases, three sulfatases, and HGSNAT. Deficiency in HGSNAT results in Sanfilippo syndrome (MPS-IIIC), a functional disorder of the brain. In mice, inactivation of the HgsNat gene leads to a mild form of MPS-IIIC and reduced litter sizes. In this study, the testes and epididymides of WT and HgsNat−/− mice at different ages, i.e., 7, 11 and 14 months were fixed with 2.5% glutaraldehyde and processed for EM analysis, or immersed in Bouin’s fixative for LM immunocytochemical studies. In Hgsnat deficient mice, some seminiferous tubules revealed highly vacuolated areas and/or major depletion of the germ cell population. Approximately 20% of the tubules were decreased in size as compared to WT mice. In WT epididymis, principal cells contained distinct dense spherical lysosomes, which contrasted the numerous empty looking vacuoles that appeared both supranuclearly and infranuclearly in knockout mice. LM immunocytochemical data revealed a specific expression of cathepsin-D and prosaposin in these vacuoles suggesting that they were lysosomal in nature. Clear cells became highly vacuolated and lacked their characteristic numerous dense lysosomes. These cells also appeared to lose contact with the lumen and be greatly increased in size. Our results show that HgsNat inactivation affects spermatogenesis and the appearance of epididymal epithelial cells, with a phenotype similar to lysosomal storage disorders.

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CANDIDATE GENES IMPLICATED IN THE GENERATION OF PHENOTYPIC VARIABILITY IN 22q11.2 DELETION SYNDROME; EXPRESSION OF Snap29 AND Scarf2

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Introduction 22q11.2 deletion syndrome (22q11.2DS) is a contiguous gene syndrome with prevalence of nearly 1 in 4000 live birth. About 90% of 22q11.2DS patients have a 3 Mb deletion of approximately 45 functional genes including SNAP29, and SCARF2, genes that have been shown to contribute to developmental syndromes, CEDNIK and VDEGS, respectively. We postulate that deletion of SNAP29 and SCARF2 contribute to phenotypic variability seen in subset of patients. Our aims are to characterize expression of these genes during development and to generate mouse models to determine where and when they are required for normal development.

Methods We used in situ hybridization and immunohistochemistry to characterize expression of Snap29 and Scarf2 throughout mouse development. Conditional mutant mouse line with mutation in Snap29 was ordered from EMMA. A patient-specific mutation in Snap29 is being generated with CRISPR/Cas technology.

Results We have found that both genes are express in the embryonic precursors of organs that are affected in human patients with mutations in these genes. In addition, expression were found in a number of additional sites not known to be affected in the patients. We are waiting for our mouse models in order to characterize their developmental roles.

Conclusion Patients carrying mutations in SNAP29 or SCARF2 have syndromes with a number of abnormalities. These two gene are deleted in 90% of patients and mouse models generated for 22q11.2DS do not include neither SNAP29 or SCARF2. Our results suggest that these two genes might contribute to abnormalities that cannot be explain by previously generated mouse models.
IDENTIFICATION OF CLAUDIN PROTEIN VARIANTS IN CHILDREN WITH CHRONIC KIDNEY DISEASE

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Congenital renal malformations are a major cause of kidney failure in children. Kidney formation begins with the differentiation of the nephric duct from the intermediate mesoderm, and the development of the nephron through reciprocal signalling between the mesenchyme and the ureteric bud epithelium. Claudins are integral tight junction transmembrane proteins that regulate the intercellular barrier and pore properties of epithelial cell layers. The C-terminal tail of claudins contain a PDZ binding motif that allows interaction with scaffolding proteins linking tight junctions to the actin cytoskeleton. The four transmembrane domains and the two extracellular loops in claudins contribute to barrier/pore formation and intercellular adhesion. Given the importance of claudins in epithelium organization, we hypothesize that claudins are necessary in the development of the kidney. To determine this, we will identify and validate protein altering sequence variants from all 24 claudin genes in 96 patients with congenital renal malformations from the NIH-sponsored CKiD (Chronic Kidney Disease) study. Recently, we identified a missense variant, A94V, in the second transmembrane domain of claudin 8 in one patient. Next, we will look at the expression and localization of this claudin variant to the tight junction, and, using the chick embryo as the animal model, observe its effect on formation of the mesonephric duct and ureteric bud branching. This study will provide a better understanding of the molecular mechanisms involved in the formation of tubular epithelium during kidney development.

Funding sources: Kidney Foundation of Canada
The retrograde flow of urine from the bladder to the kidneys, vesicoureteral reflux (VUR), results from developmental defects in the ureterovesical junction (UVJ). Perturbations in the extracellular matrix components of the UVJ, and mutations in Tenascin-XB, which encodes for a regulatory matrix protein, are associated with VUR. The inbred C3H/HeJ (C3H) mouse is a model of recessively inherited VUR that maps to the proximal end of chromosome 12—the Vurm1 locus. Putative disease-causing variants in ECM-related genes such as Itgb8, Itgbp1, Lmb1, Fbln5 have been identified within Vurm1. In both refluxing and non-refluxing (C57Bl/6J) mice, expression analysis of fibrillary collagens in the urinary tract shows that collagen fibres grow in length and thickness with age with a preponderance of COL1 noted in adult mice, while elastic fibres are sparse and interwoven between collagen fibres in both newborn and adult. Starting at the newborn stage, the relative amount of fibrillary collagens in the ureter and the bladder of C3H mice is significantly higher than in non-refluxing mice and the musculature of the bladder exhibits degenerative changes that continue to adulthood. A stiffer bladder and UVJ due to an increase in their collagen content could explain VUR in C3H mice. We sequenced tenascin XB in a cohort of children with VUR and have identified rare TNXB variants, including E1251G and M2053V. These variants occur in an evolutionary conserved region of the protein important for interaction with collagens and integrins. **Conclusion:** Our results suggest there is a link between ECM perturbations and VUR.

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ROLE OF ERK1/2 IN BOVINE OVULATION

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The most common reason a dairy cow is removed from the herd is infertility, which can be attributed to ovarian dysfunction. It is currently unknown whether or not extracellular-regulated kinases 1 and 2 (ERK1/2), a fundamental player in murine ovulation, plays an equally vital role in bovine ovulation. Cows were synchronized and 30 minutes prior to GnRH stimulation their dominant follicle was treated, by intra-follicular microinjection, with a vehicle or PD0325901 (ERK1/2 inhibitor; 1µM, 10µM or 50 µM). Transrectal ultrasonography revealed that cows treated with 50 µM PD0325901 failed to ovulate. Next, cows treated with a vehicle or ERK1/2 inhibitor (50 µM) were ovariectomized either prior to 0h or at 6h after GnRH to collect granulosa and theca cells of ovulating follicles. Transcript levels of EGR1, EREG and TNFAIP6 were higher (P < 0.05) at 6h post-GnRH in granulosa cells of vehicle treated follicles compared to 0h follicles. However, these transcripts were lower (P < 0.05) at 6h in granulosa cells of PD0325901 follicles compared to 6h vehicle treated follicles. In theca cells, TIMP1 levels were lower (P < 0.05) at 6h post-GnRH in inhibitor treated follicles compared to 6h vehicle treated follicles. STAR mRNA abundance in theca cells was higher (P < 0.05) at 6h post-GnRH in inhibitor treated follicles compared to 6h vehicle treated follicles. Similar to theca cells, STAR tended to be higher at 6h post-GnRH in granulosa cells from PD0325901 inhibited follicles compared to vehicle treated follicles. Thus, similar to mice, inhibition of ERK1/2 signaling abolishes ovulation in cows.
DOES MUTATION IN HUMAN BINDER OF SPERM GENE AFFECT MALE FERTILITY?

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Binder of Sperm (BSP) genes are known to promote sperm capacitation through binding to sperm membrane shown by in vitro studies in bovine as well as in human by our group. To further elucidate role of BSPs in capacitation, we will study the effect of gene mutations already identified in the NCBI SNPs database. We generated four frame shift mutations using site directed mutagenesis on wild-type (WT) recombinant protein to analyse function and structure of BSPH1 protein in depth. These proteins were expressed with their own expression patterns and purified on His bind column. The capacity of rec-BSPH1 mutants to bind to heparin was also tested by affinity chromatography. Next, we will evaluate the effect of these mutants on different sperm functions such as capacitation assay. Protein sequence of human Binder of Sperm Homolog 1 (BSPH1) was analysed with Snap 2 software and Consurf server to dig out the best candidate to study. Furthermore, we will identify new mutations in human BSPH1 gene through next generation sequencing and bioinformatics tools to verify if there is a correlation with idiopathic male infertility. Finally, we will define problems linked with the expression or regulation of BSPH1 gene that would not be identified in the sequencing of coding regions by immunoprecipitation using monoclonal antibody. Identification of infertility due to mutation(s) related to BSP genes could provide a diagnostic tool and therapeutic applications. Additionally, this research could be a major breakthrough in the field leading to potential development of a male contraceptive.

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DNA FRAGMENTATION ASSOCIATED WITH CRYOPRESERVATION OF HUMAN TESTICULAR SPERM

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Purpose: Although ejaculated sperm is the most common source of sperm for cryopreservation, for patients with poor ejaculated sperm, azoospermia or those who will undergo surgery in the testis, testicular sperm may also be cryopreserved. The objective of our study was to evaluate the impact of cryopreservation on the sperm chromatin quality of testicular sperm in various groups of patients.

Experimental approach: Twenty patients were subdivided in four groups: 1) normal spermatogenesis, 2) varicocele, 3) testicular cancer, and 4) abnormal sperm chromatin structure in ejaculated sperm. Terminal deoxynucleotidyl transferase (TdT) dUTP Nick-End Labeling (TUNEL) assay was used to quantify DNA fragmentation. Samples were evaluated both fresh and after being frozen for a minimum of one week.

Results: Fresh testicular sperm had a mean DNA fragmentation of 27.2% (SD 12.1%) with a statistically significant increase to 43.4% (SD 15.3%) following cryopreservation (p <0.001). Varicocele, and testis cancer patients did not achieve statistically significant changes in DNA fragmentation (p = 0.34, and N/A) after freezing. Normal (23.0% vs 34.4%, p = 0.02) and patients with high DNA fragmentation in ejaculated sperm (30.5% vs 54.9%, p = 0.01) had a significant increase in the extent of DNA fragmentation after freezing.

Conclusion: Cryopreservation may damage or worsen the quality of testicular sperm in some patients, particularly those with otherwise normal testicles and those who already have abnormal sperm DNA in the ejaculated sperm. Further studies with large samples size are required to confirm our preliminary findings and to evaluate their impact on reproductive outcomes.

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Adequate zinc levels are required for proper cellular functions and for male germ cell development. Zinc transport is accomplished by two families of zinc transporters, the ZnTs and the ZnTs, that increase and decrease cytosolic zinc levels, respectively. However, very little is known about zinc transport in the testis. Furthermore, whether cytotoxic agents such as cyclophosphamide (CPA), a known male germ cell toxicant, can affect zinc transport and homeostasis is unknown. Using whole genome microarray, we examined zinc transporter expression and zinc transport in pachytene spermatocytes (PS) and round spermatids (RS) isolated from Sprague Dawley rats gavaged with saline (control) or CPA (6 mg/kg) for 4 weeks. We observed differences in the expression of members of the ZnT and ZIP families in purified populations of PS and RS. Using a fluorescent zinc probe and live cell imaging, we also observed that RS accumulate more zinc over time than PS. The expression of many zinc binding genes was altered after CPA treatment. Interestingly, we found that the expression of ZIP5 and ZIP14 was increased in PS from CPA-treated rats, but not in RS. This up-regulation led to an increase in zinc uptake in PS, but not in RS, from treated animals compared to controls. These data suggest that CPA treatment may alter zinc homeostasis in male germ cells leading to an increased need for zinc. Altered zinc homeostasis may disrupt proper germ cell development and contribute to infertility and effects on progeny.

This study is supported by CIHR.
IN OVO POTENCY OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) IN CHICKEN EMBRYOS

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Dioxin-like compounds are known developmental toxicants in wildlife. One challenge for risk assessment for these chemicals is that large differences in potency exist among individual congeners and species. In fish, the in vitro potency of PAHs is predictive of the in vivo potency. However, very little data exist on the relative potency (LD50 values) of PAHs in birds. We determined the embryolethality of 5 polycyclic aromatic hydrocarbons (PAHs) congeners (known to be AHR agonists in birds) in developing chicken embryos (Gallus gallus domesticus). A dose-dependent increase in mortality was observed for all congeners tested. The rank order potency based on LD50 values was as follows: dibenz[ah]anthracene > benzo[k]fluoranthene > indeno[1,2,3-cd]pyrene > benzo[a]pyrene > benz[a]anthracene. This is consistent with the relative order of potency for induction of ethoxyresorufin-O-deethylase (EROD) activity in cultured hepatocytes. PAH exposure did not affect weight, pipping time (time from beginning of incubation to pipping) or induce significant developmental abnormalities. This study is the first to use environmentally relevant dosing scenarios to determine LD50 values and rank the potency of PAHs congeners in birds. This data may be useful for further research exploring sub-lethal effects of PAHs in avian embryos.

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THE EFFECTS OF GESTATIONAL AND LACTATIONAL EXPOSURE TO REPLACEMENT PLASTICIZERS ON THE DEVELOPMENT OF MALE SPRAGUE-DAWLEY RATS

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* equal contribution

Phthalates account for 75% of global plasticizer use. Phthalates can be found in a variety of products, including construction materials, cosmetics, medical devices, and other products made from polyvinyl chloride. Their widespread use and the ability of these chemicals to leach has made di-(2-ethylhexyl) phthalate (DEHP) a ubiquitous environmental contaminant. Evidence in animal models has identified these chemicals as endocrine disruptors with anti-androgenic properties. Several alternatives were developed to meet government regulations, consumer demand, and manufacturing requirements; 1,2-Cyclohexane dicarboxylic acid diisononyl ester (DINCH) is one such replacement plasticizer. Our group has screened two replacements (1,4 butanediol dibenzoate (BDB) and dioctyl succinate (DOS)) in a 28-day acute toxicity study and found no systemic toxicity; thus allowing us to proceed to a for further investigation in a gestational- and lactational exposure study.

Timed-pregnant Sprague-Dawley rats were treated with 30 mg/kg or 300 mg/kg BDB, DOS, DEHP, or DINCH from gestation day (GD) 8 to postnatal day (PND) 21. Litters were culled at PND 3, and pups were necropsied at PND 21, 46, and 90. Select reproductive and vital organs were collected for histology and RNA while serum was collected for biomarker analysis. There were no significant differences among treatment groups in pregnancy outcome, reproductive organ weight, preputial separation, nipple retention index, serum testosterone, LH or FSH levels. There was a decrease in anogenital index at PND 3, and an increase in haemorrhagic testes at PND 8 with 300 mg/kg DEHP. Based on the data collected from these experiments, our novel green plasticizers are viable phthalate replacements.

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A COMPARISON OF THE EFFECTS OF BROMINATED DIPHENYL ETHER-47 (BDE-47) AND ISOPROPYLATED TRIPHENYL PHOSPHATE (IPPP) ON MURINE LIMB DEVELOPMENT IN VITRO

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The toxicity of brominated flame retardants (BFRs) such as BDE-47 has led to their replacement with organophosphate flame retardants (OPFRs) such as IPPP. Indeed, environmental levels of OPFRs are now comparable to or greater than those of BFRs. Therefore, evaluation of their potential toxicity is crucial.

Previous studies have shown that in utero exposure to flame retardants may have adverse effects on skeletal development. The goal of this study was to compare the effects of BDE-47 and IPPP on limb and bone formation using the murine limb bud culture system. Limb buds excised from CD-1 embryos on gestation day 13 were cultured for 6 days in the presence of vehicle (DMSO), BDE-47 (10 or 50 μM), or IPPP (1, 3, 10 or 50 μM).

Limb morphology scores revealed that 50 μM BDE-47 significantly decreased the number of phalangeal cartilage condensations that developed across all digits compared to controls. In comparison, IPPP had a similar effect at only 3 μM and higher concentrations stunted differentiation throughout the limb. To monitor effects on the main stages of endochondral ossification, we used mice expressing fluorescently tagged COL2A1 (chondrogenesis), COL10A1 (early osteogenesis), and COL1A1 (late osteogenesis). BDE-47 had no marked effect at the concentrations tested. In contrast, COL2A1 expression was reduced after exposure to 10 or 50 μM IPPP; COL10A1 and COL1A1 were reduced by 3 μM IPPP and largely absent at higher concentrations. Thus, the impact of IPPP on limb patterning and bone formation in vitro is greater than that of BDE-47.

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Thank you for attending our Research Day. We’d like to hear your impression on the various aspects of the event. We will use your responses to help tailor our next Research Day to deliver an enjoyable experience for all attendees.

Using a scale of 1 to 5 with 1 indicating well below your expectations and 5 well above your expectations, please rate each of the following:

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Comments/Suggestions:
Nouvelles avancées en reproduction et développement

Journée de recherche du Centre de recherche en reproduction et développement (CRRD) à McGill

Mardi le 31 mai 2016

FORMULAIRE D’ÉVALUATION

Merci d’assister à notre journée de recherche. Nous aimerions avoir vos commentaires sur différents aspects de l’événement. Vos réponses et commentaires nous aideront à améliorer notre prochaine journée de recherche.

Sur une échelle de 1 à 5, avec 1 étant « pas du tout satisfait » et 5 étant « très satisfait », veuillez indiquer votre degré de satisfaction concernant les aspects suivants :

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