Centre for Research in Reproduction and Development
Centre de recherche en reproduction et développement

BREAKTHROUGHS IN REPRODUCTION AND DEVELOPMENT

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NOUVELLES AVANCÉES EN REPRODUCTION ET DÉVELOPPEMENT

Research Day

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

Tuesday, May 16, 2017

McGill New Residence Hall
3625, avenue du Parc
Montréal, Québec

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Tuesday, May 16, 2017
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8:00-8:45 Registration and Coffee / Poster set-up

8:45-9:00 Opening Remarks: **Dr. Daniel Bernard**

9:00-9:45 Trainee Oral Presentations Session I (Chairs: Matthew Ford and Deepak Tanwar)

O-01. Angus Macaulay, *"MCAK mediated error correction in the early embryo prevents mosaic aneuploidy"*

O-02. Sophia Rahimi, *"Effect of folic acid supplementation on adverse morphological and epigenetic outcomes in offspring conceived using assisted reproduction"*

O-03. Keith Siklenka, *"Histone H3K4me3 is implicated in paternal epigenetic inheritance"*

9:45-11:00 Morning Break and Poster Session I (judging 10-11)

P-01. Emilie Brûlé, *"Does IGSF1 play a role in thyroid hormone transport?"*

P-03. Luisina Ongaro, *"Activins stimulate human follicle-stimulating hormone ß (Fshb) expression via a FOXL2-dependent mechanism in pituitaries of transgenic mice"*

P-05. Chirine Toufaily, *"Gαq/11 and Gαs play important and distinct roles in gonadotrope cells in vivo"*

P-07. Brandon Vaz, *"The role of X-chromosome asynapsis in the elimination of murine oocytes"*

P-09. Adel Moawad, *"Critical role of peroxiredoxins in protecting mouse spermatozoa against oxidative stress"*

P-11. Hamed Heidari Vala, *"Binder of Sperm (BSP) protein can interfere in sperm-egg interaction and fertilization"*

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P-19. Lia Mara Gomes Paim, *"Binucleation fails to activate a tetraploidy checkpoint and instead causes segregation errors in the preimplantation embryo"*

P-21. Amanda Baumholtz, *"Claudins regulate cell shape and localization of signaling proteins at the apical cell surface during neural tube closure"*

P-23. Karina Gutierrez, *"Effect of the Injection Time and Inhibition of DNA Repair on Genome Editing Efficiency in Porcine Embryos Produced by Activation, IVF, ICSI and SCNT"*

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P-35. Rose Ghemrawi, *"Determining the links between body mass index, folate, homocysteine and fertility parameters in men"*
P-37. Emily Boulanger, *"Effects of early life exposure to contaminated sediment in fish"*
P-39. Anne Marie Downey, *"Zinc supplementation reduces cyclophosphamide induced oxidative stress and DNA damage in male germ cells"*
P-41. Katie Teng, *"Investigation loss-of-Lkb1 function in oviductal epithelium and its role in facilitating the initiation of high-grade serous ovarian carcinoma (HGSOC)"*

11:00-11:45 **Dr. Marisa Bartolomei, Co-Director, Epigenetics Program, University of Pennsylvania School of Medicine**, *"Epigenetic reprogramming of genomic imprints"* – introduced by Dr. Jacquetta Trasler

11:45-12:30 Trainee Oral Presentations Session II (Chairs: Olusola Sotunde and Shahrzad Ghazisaeidi)
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O-06. Yining Li, *"Conditional deletion of FOXL2 and SMAD4 in gonadotropes of adult mice impairs FSH production and fertility"*

12:30-13:30 Lunch (visit sponsor booths)

13:30-14:15 **Dr. Liliana Attisano**, **Department of Biochemistry, University of Toronto**, *"Identifying new targets for therapeutic intervention in the tumor-promoting Hippo pathway"* – introduced by Dr. Aimee Ryan

14:15-15:30 Afternoon Break and Poster Session II (judging 14:30-15:30)
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15:30-16:15 **Dr. Marjorie Dixon**, **CEO & Medical Director, Anova Fertility & Reproductive Health, Toronto**, *"Assisted reproductive technologies and oocyte physiology in the year 2017: What role do the mitochondria play?"* – introduced by Dr. Daniel Bernard

16:15-16:30 Concluding Remarks: **Dr. Daniel Bernard**

16:30-17:30 Awards Presentation & Reception / Take down posters

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NOUVELLES AVANCÉES EN REPRODUCTION ET DÉVELOPPEMENT

Journée de recherche 2017

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le mardi 16 mai 2017
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8h Inscription et café / Installation des affiches

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12h30 Dîner (visitez les tables de sponsor)

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15h30 **Dr. Marjorie Dixon**, **CEO & Medical Director, Anova Fertility & Reproductive Health, Toronto**, *"Assisted reproductive technologies and oocyte physiology in the year 2017: What role do the mitochondria play?"* – présentée par Dr. Daniel Bernard

16h15 Mots de conclusion : **Dr. Daniel Bernard**

16h30-17h30 Présentation des prix, réception et démontage des affiches

MARISA BARTOLOMEI

UNIVERSITY OF PENNSYLVANIA PERELMAN SCHOOL OF MEDICINE

EPIGENETIC REPROGRAMMING OF GENOMIC IMPRINTS

Imprinted genes are expressed from a single parental allele and most reside in clusters that are located throughout the mammalian genome. The clusters typically contain an imprinting control region (ICR), which harbors allele-specific methylation and governs imprinting. Although most imprinted clusters use lncRNAs to regulate imprinted gene expression, a few are regulated by CTCF. One such cluster harbors the H19 and Igf2 imprinted genes, and is controlled by an ICR that contains multiple CTCF binding sites. Gain of maternal methylation and loss of paternal hypermethylation of the H19/IGF2 ICR are associated with the human growth disorders Beckwith-Wiedemann Syndrome and Silver-Russell Syndrome, respectively. Using gene targeting and genome editing, we have generated cell lines and mice to study imprinting mechanisms and model the epigenetic mutations in human syndromes. We have also studied the mechanism governing imprint establishment in the germline using mice harboring mutations in epigenetic regulators.

Brief Bio: Marisa Bartolomei is a Professor of Cell & Developmental Biology and co-Director of the Epigenetics Program at the University of Pennsylvania Perelman School of Medicine. Marisa S. Bartolomei received her BS in Biochemistry at the University of Maryland and then obtained her PhD from the Johns Hopkins University School of Medicine under the guidance of Dr. Jeffry Corden. She trained as a postdoctoral fellow with Dr. Shirley Tilghman at Princeton University. In 1993, Dr. Bartolomei was appointed as an Assistant Professor at the University of Pennsylvania and was promoted to Associate Professor with tenure in 1999 and Professor in 2006. In 2006, Dr. Bartolomei received the Society for Women's Health Research Medtronics Prize for Contributions to Women's Health. In 2011, Dr. Bartolomei received the Jane Glick Graduate School Teaching Award for the University of Pennsylvania School of Medicine and a MERIT award. She was elected as a Fellow of the American Association for the Advancement of Science in 2014 and is recipient of the 2017 Genetics Society Medal from the UK Genetics Society.

LILIANA ATTISANO

DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF TORONTO

IDENTIFYING NEW TARGETS FOR THERAPEUTIC INTERVENTION IN THE TUMOR-PROMOTING HIPPO PATHWAY

The Hippo signalling pathway is a key regulator of tissue growth and organogenesis. Pathway inactivation is a common feature in numerous cancers, yet, mutations in pathway components are relatively rare. The pathway is comprised of a core MST/LATS kinase cassette that phosphorylates and promotes cytoplasmic localization of the transcriptional regulators, TAZ and YAP. To uncover novel Hippo pathway regulators, we have conducted multidimensional high throughput screens involving LUMIER-based protein-protein interaction mapping, functional cDNA overexpression and siRNA knockdown assays using TAZ/YAP transcriptional reporters and imaging-based subcellular localization assays. These efforts have uncovered positive regulators such as the scaffolding protein betaPIX and several kinases that function to block Hippo pathway activity, including members of the MARK kinase family. The molecular mechanisms and contribution to tumorigenic properties will be presented. Thus, our screens have identified both positive and negative regulators of the Hippo pathway that may that may serve as useful targets for therapeutic intervention.

Brief Bio: Liliana Attisano is currently a Professor in the Department of Biochemistry, Donnelly Centre, at the University of Toronto. She currently holds a Canada Research Chair in Signalling Networks in Cancer. The Attisano lab is focused on studying the molecular events that underlie signalling cascades and in understanding how cells interpret contextual cues to control complex biological responses. A current area of interest is on understanding how the Hippo tissue growth control pathway interacts with and modulates responses to morphogens like Wnt and TGFbeta, whose disruption is associated with numerous human cancers. For this, the lab employs a high-throughput integrated screening approach that involves analysis of mammalian-cell based protein-protein interactions using LUMIER method combined with parallel functional assays to assess the effects of overexpression, siRNA-mediated knockdown or chemical compounds on signalling outcome. Our recent analysis has revealed crosstalk between the Wnt and Hippo pathways and has uncovered novel regulators of the Hippo pathway.

MARJORIE DIXON

ANOVA FERTILITY & REPRODUCTIVE HEALTH

ASSISTED REPRODUCTIVE TECHNOLOGIES AND OOCYTE PHYSIOLOGY IN THE YEAR 2017: WHAT ROLE DO THE MITOCHONDRIA PLAY?

There has been a huge paradigm shift in society where women are now delaying childbirth on a regular basis. In fact, there is a significant trend to first delivery after the age of 35 years in North America. In striking contrast, though we have made enormous strides in the clinical realm with advances in assisted reproductive technologies, we have not yet managed to change the evolution of the female ovary. Ovarian aging is a conundrum that is plaguing reproductive scientists and is the focus of significant research hypotheses.

- What role do the mitochondria play? Is there a genetic basis for these processes?
- Are we on the verge of an assisted reproductive revolution?

We will review the current literature and see what evidence exists for some of the current laboratory/ clinical practices. This will be a preamble into the future of human reproductive biology.

Brief Bio: Dr. Marjorie E. Dixon is a graduate of McGill University's School of Medicine. Following her initial postgraduate training at the University of Toronto in obstetrics and gynecology, Dr. Dixon completed her postgraduate training at the University of Vermont with a three-year subspecialty at the American Board of Obstetrics and Gynecology accredited fellowship in reproductive endocrinology and infertility.

She joined the department of obstetrics and gynecology at Sunnybrook Health Sciences Centre more than a decade ago, and she continues to be an involved member today. In 2005, she was appointed assistant professor at the University of Toronto, where she actively participates in the education of medical students and residents.

As an advisory board member of the Ministry of Health, Dr. Dixon played an instrumental role in the approval of the 2015 IVF Ontario Funding Program. She is an invited speaker at both national and international conferences in her field of reproductive endocrinology and infertility and she continuously advocates for women's health.

Since 2010, on the last Thursday of every month, Dr. Dixon has been a regular feature on the CityLine television program, educating Canada on women's health.

Dr. Dixon's passion for educating women on their reproductive health encouraged her to open Anova Fertility & Reproductive Health in 2016, the newest full-service IVF centre in Canada exclusively focused on reproductive medicine and women's health.

For Dr. Marjorie E. Dixon's full bio click here.

ORAL PRESENTATIONS

ORAL 1

MCAK MEDIATED ERROR CORRECTION IN THE EARLY EMBRYO PREVENTS MOSAIC ANEUPLOIDY

Angus Macaulay and Greg FitzHarris

Département d'obstetrique-gynécologie, CRCHUM, Université de Montréal

Early embryonic divisions are surprisingly error prone and result in chromosomally mosaic embryos, however the precise dynamics of how chromosomes are organized in mitosis in this setting is yet to be addressed. One important protein in canonical mitoses is mitotic centromere associated kinesin (MCAK), an atypical motor protein known to contribute to faithful chromosome segregation and effective chromosome congression in somatic cells. MCAK destabilizes microtubules that form incorrect attachments to kinetochores. The mechanisms that govern protective pathways in blastomeres often display subtle differences from their somatic cell counterparts. Therefore we set out to establish chromosome dynamics, and characterize the role of MCAK in the murine pre-implantation embryo.

Live-cell imaging in 4D of centromeres in early embryonic divisions revealed mitosis to be a three-stage process consisting of (1) the outer sphere of centromeres rapidly invading the center of the yet unorganized spindle at the time of nuclear envelope breakdown, (2) a period of relative spatial disorganization of centromeres, (3) a progressive congression phase resulting in metaphase alignment. MCAK knockdown caused increased rates of micronuclei formation and lessened overall cell number at the morula stage. The impact of MCAK removal was even more obvious when cell division was experimentally perturbed, following a pulse of the kinesin-5 inhibitor monastrol. MCAK overexpression minimized the severity of metaphase alignment errors. MCAK depletion and overexpression had reciprocal impacts on the duration of spindle reassembly after monastrol washout.

Early embryo mitosis is a complex multistep process, requiring tight MCAK regulation. The importance of MCAK for faithful chromosome segregation is particularly evident when spindle function is perturbed.

Funding sources: The FitzHarris Lab is supported By CIHR, NSERC, and the Jean-Louis Levesque foundation. Angus Macaulay is supported by an NSERC Fellowship.

ORAL 2

EFFECT OF FOLIC ACID SUPPLEMENTATION ON ADVERSE MORPHOLOGICAL AND EPIGENETIC OUTCOMES IN OFFSPRING CONCEIVED USING ASSISTED REPRODUCTION

Sophia Rahimi[1,2], Josée Martel[1], and Jacquetta Trasler[1,2,3]

[1]Research Institute of the McGill University Health Centre, Montreal, Quebec, Canada
[2]Department of Human Genetics, McGill University, Montreal, Quebec, Canada
[3]Departments of Pharmacology & Therapeutics and Pediatrics, McGill University, Montreal, Quebec, Canada

Approximately 1-6% of children are conceived using assisted reproductive technologies (ART); however, adverse outcomes have been associated with their use. These procedures have been shown to alter epigenetic programming events during germ cell and early embryo development. Importantly, the availability of methyl donors such as dietary folate is essential for proper establishment of DNA methylation profiles during these stages of development. The objective of my research is to determine whether folic acid supplementation can prevent birth defects and epigenetic abnormalities associated with ART. Female mice were fed control diet (2 mg folic acid/kg; CD), 4-fold folic acid-supplemented diet (8 mg folic acid/kg; 4FASD) or 10-fold folic acid-supplemented diet (20 mg folic acid/kg; 10FASD) six weeks prior to assisted reproduction and throughout gestation. The proportion of developmentally delayed embryos was significantly lower in the 4FASD group. In addition, we noted a trend towards increased embryonic abnormalities in the 10FASD group when compared to the 4FASD group. We also examined imprinted gene methylation in midgestation placentas by bisulfite pyrosequencing. Compared to naturally-mated mice fed the control diet (NAT CD), all ART groups demonstrated significantly lower methylation and increased variance at all CpGs within *Snrpn*, *H19* and *Peg1* differentially methylated regions. Overall, our results suggest that folic acid supplementation in pregnancies achieved by ART affects embryonic outcomes in a dose-dependent manner whereas imprinted gene methylation in placentas is not affected. 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 and CRRD.

ORAL 3

HISTONE H3K4ME3 IS IMPLICATED IN PATERNAL EPIGENETIC INHERITANCE

Keith Siklenka[1], Christine Lafleur[2], Sarah Kimmins[1,2]

[1] Department of Pharmacology and Therapeutics, McGill University, Montreal, QC, Canada
[2] Department of Animal Science, McGill University, Ste-Anne-de-Bellevue, QC, Canada

Introduction: Paternal epigenetic inheritance has been described in human cohorts and animal models ranging from worm to mouse. Despite these observations, the underlying molecular mechanisms of inheritance remain unknown. Recently we've shown that disruption of histone-H3 lysine 4 di-methylation (H3K4me2) by overexpression of KDM1A in testis leads to reduced survivability and abnormal offspring development. Remarkably these effects persisted transgenerationally, but the sperm chromatin alterations underlying the transgenerational phenomena remained unresolved. It was clear that H3K4me2 was not directly involved as nonTG sires had normal sperm profiles for this mark despite similar offspring phenotypes as TG sires.
We **hypothesized** that KDM1A overexpression, and resulting H3K4me2 reduction, would also influence surrounding chromatin. Therefore, our **objective** was to examine the related histone marks H3K27me3 and H3K4me3 in TG and nonTG sperm and identify altered histone states associated with epigenetic inheritance. **Methods:** We prepared native chromatin from pooled sperm of C57Bl/6, TG and nonTG mice (n = 3 pooled/group) and performed ChIP-seq using antibodies against H3K27me3 (1 replicate /group) or H3K4me3 (3 replicates / group). Data sets were quantitatively analyzed with R/Bioconductor packages csaw and DiffBind. **Results:** Basic quantification of H3K27me3 failed to detect differences in enrichment or distribution between genotypes and generations. However, quantification of H3K4me3 in TG-sperm revealed over 2000 TSS had increased methylation when compared to control. Strikingly, in nonTG-sperm, 200 genes were elevated over control, but 161 (80%) were common between TG and nonTG males. Therefore, we describe a set genes with paternally inherited histone-modifications capable of escaping epigenetic reprogramming.

Funding sources: Research supported by CIHR

ORAL 4

LONG-TERM EFFECTS OF IN UTERO AND LACTATIONAL EXPOSURE TO NEW GENERATION GREEN PLASTICIZERS ON MALE REPRODUCTIVE FUNCTION: A COMPARATIVE STUDY WITH DEHP

Océane Albert[1], Thomas C. Nardelli[1], Claudia Lalancette[1], Barbara F. Hales[1], Bernard Robaire[1,2]

[1]Department of Pharmacology and Therapeutics, McGill University, Montreal, Quebec, Canada
[2]Department of Obstetrics and Gynecology, McGill University, Montreal, Quebec, Canada

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; 1,4-butanediol dibenzoate (BDD) and dioctyl succinate (DOS) displayed the most innocuous profiles after *in vitro* testing on multiple cell lines and were consequently selected for *in vivo* studies. We hypothesized that BDD and DOS exert fewer endocrine disrupting effects than DEHP on the adult male rat reproductive system. Nine groups of Sprague Dawley dams were dosed daily with corn oil, BDD or DOS (30 or 300 mg/kg) from gestational day 8 to postnatal day 21 (PND21); DEHP and DINCH (currently used as a replacement for DEHP) served as reference compounds. At PND46 and 90, BDB and DOS did not significantly affect the male pups' genital tract organ weights, serum LH, FSH or testosterone concentrations. Sperm motility at PND90 remained unaffected. Testicular gene expression was most affected by exposure to the high DEHP dose; 50 transcripts displayed significant differential expression (> 1.5-fold change). The nuclear receptor Nr5a2, involved in reverse cholesterol transport and steroidogenesis, was the most drastically affected transcript, with a 7.3-fold upregulation after exposure to 30 mg/kg DEHP. While further experiments are needed to fully assess the consequences of exposure to BDB and DOS, the evidence obtained to date indicates that these candidate compounds display fewer long-term effects on testicular function than DEHP.

Funding sources: These studies were supported by the CIHR Institute of Human Development, Child and Youth Health, the CIHR REDIH Training Program, and the Fonds de la Recherche du Québec en Santé.

ORAL 5

A COMPARISON OF THE EFFECTS OF BDE-47 AND FOUR ORGANOPHOSPHATE ESTER (OPE) FLAME RETARDANTS ON ENDOCHONDRAL OSSIFICATION IN VITRO

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Flame retardants are added to consumer goods to slow burning. Brominated flame retardants (BFRs) have been shown to be persistent, bioaccumulative and toxic. Thus, they have largely been replaced with organophosphate esters (OPEs). Despite detectable levels of OPEs now surpassing those of BFRs, we know little about their safety.

Developmental BFR exposure has been associated with adverse effects on endochondral ossification. The aim of this study was to compare the effects of a predominant BFR (BDE-47) and four common OPEs (TPHP, TMPP, IPPP, BPDP) on bone development using the limb bud culture system. Limb buds of gestational day 13 mouse embryos were cultured in the presence of vehicle, BDE-47 (10 or 50 μM), or each OPE (1, 3 or 10 μM).

Morphological scores revealed that 50 μM BDE-47 significantly decreased the number of cartilage condensations that developed across all digits compared to controls. In contrast, 1 μM of each OPE was sufficient to produced similar or greater effects. To examine changes in endochondral ossification progression, we used mice expressing fluorescently tagged COL2A1 (chondrogenesis), COL10A1 (early osteogenesis), and COL1A1 (late osteogenesis). While BDE-47 had no notable effect on any marker at the concentrations tested, 3 μM of each OPE reduced COL10A1 and COL1A1 expression. Thus, all four OPEs tested were more detrimental to endochondral ossification in limb bud cultures than BDE-47. In the context of bone health, at least some OPEs may be more toxic than their brominated predecessors.

Funding sources: CIHR, RQR, CRRD, McGill (GEF, Alexander McFee Fellowship).

ORAL 6

CONDITIONAL DELETION OF FOXL2 AND SMAD4 IN GONADOTROPES OF ADULT MICE IMPAIRS FSH PRODUCTION AND FERTILITY

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Follicle-stimulating hormone (FSH) regulates ovarian follicle development in females and spermatogenesis in males. Activins stimulate FSH synthesis in pituitary gonadotropes by regulating transcription of the FSHβ subunit gene (*Fshb*) via the transcription factors SMAD4 and FOXL2. Gonadotrope-specific deletion of both *Smad4* and *Foxl2* using Cre-lox produces mice that are profoundly FSH-deficient, sterile (females), or oligozoospermic (males). However, the Cre allele used previously is first active during pituitary development. Thus, it is unclear whether the diminished FSH synthesis reflects impaired gonadotrope development, the necessity for SMAD4 and FOXL2 in adulthood. To discriminate between these possibilities, we generated a new mouse line that enabled the inducible deletion of *Smad4* and *Foxl2* in gonadotropes of adult mice. We compared strains of mice carrying floxed alleles for both *Smad4* and *Foxl2* with (experimental) or without (control) the inducible Cre allele. Mice of each genotype were treated with tamoxifen or with oil vehicle. Prior to treatment, all females showed normal estrous cyclicity, and fertility, suggesting no precocious Cre activity under physiologic conditions. One month after tamoxifen injections, experimental females became sub-fertile or infertile. Ovarian histology revealed arrested follicular development at the early antral stage and the absence of corpora lutea. In contrast, fertility remained intact in all other groups. Indeed, pituitary *Fshb* mRNA and serum FSH levels were reduced by ~70% following tamoxifen treatment in both female and male experimental mice. However, spermatogenesis was unaffected. Collectively, these 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.

POSTER PRESENTATIONS

Poster Session Schedule

CRRD Research Day 2017

BREAKTHROUGHS IN REPRODUCTION AND DEVELOPMENT

May 16th 2017

Presentation Time	Group 2 Presenter	Group 3 Presenter	Group 4 Presenter	Group 5 Presenter	Group 6 Presenter	Group 7 Presenter
10:00-10:10	P-01 Brûlé, Emile (Bernard)	P-07 Vaz, Brandon (Taketo)	P-27 Tokhmafshan, Fatima (Gupta)	P-19 Gomes Paim, Lia (FitzHarris)	P-23 Gutierrez, Karina (Bordignon)	P-35 Ghemrawi, Rose (Kimmins)
10:15-10:25	P-13 Priotto de Macedo, Mariana (Bordignon)	P-17 St-Yves, Audrey (Duggavathi)	P-31 Pépin, Anne-Sophie (Kimmins)	P-21 Baumholtz, Amanada (Ryan)	P-39 Downey, Anne Marie (Robaire)	P-05 Toufaily, Chirine (Bernard)
10:30-10:40	P-15 Saini, Deepak (Yamanaka)	P-41 Teng, Katie (Yamanaka)	P-37 Boulanger, Emily (Head)	P-33 Madogwe, Ejimedo (Duggavathi)		P-03 Ongaro Gambino, Luisina (Bernard)
10:45-10:55	P-25 Maunsell, Helen (Yamanaka)					
15:00-15:10	P-16 La Charité-Harbec, Simon (Gupta)	P-42 Harwalkar, Keerthana (Yamanaka)	P-04 Kim, Christine (Bernard)	P-26 Keser, Vafa (Jerome-Majewska)	P-22 Vázquez-Diez, Cayetana (FitzHarris)	P-10 Fernandez, Maria Celia (O'Flaherty)
15:15-15:25	P-24 Chan, Wesley (Jerome-Majewska)	P-32 Tanwar, Deepak (Kimmins)	P-14 Kwong, Aaron (Yamanaka)	P-18 Gamero, Enrique (Ryan)	P-28 Nguyen, Ngoc Minh Phuong (Slim)	P-34 Karahan, Gurbet (Trasler)
15:30-15:40	P-36 Allais, Adélaïde (Robaire)	P-12 Laverde, Maria (Gupta)	P-38 Hanas, Ashley (Head)	P-06 Schuermann, Yasmin (Duggavathi)	P-40 Ghazisaeidi, Shahrzad (Culty)	

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, from 10:00-11:00 and 15:00-16:00, but will not be judged because they either do not fall in one of the three categories or are from non-member labs.

P-02 Campioli, Enrico (Papadopoulos)
P-08 Sabouhi, Samin (Manjunath)
P-09 Moawad, Adel (O'Flaherty)
P-11 Heideri Vala, (Manjunath)
P-20 Eskandari-Shahraki, Marzieh (Puttaswamy)
P-29 Bertrand-Lehouillier, Virginie (McGraw)
P-30 Legault, Lisa-Marie (McGraw)

We thank ALL presenters for participating!

POSTER 1

DOES IGSF1 PLAY A ROLE IN THYROID HORMONE TRANSPORT?

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The hypothalamic-pituitary-thyroid axis regulates growth, development, and metabolism. In this axis, hypothalamic thyrotropin-releasing hormone (TRH) stimulates the secretion of pituitary thyroid-stimulating hormone (TSH). TSH stimulates thyroid hormone (TH) production by the thyroid gland. THs negatively regulate expression of the TSH subunits and TRH receptor (Trhr1) in pituitary thyrotrope cells. We recently reported that mutations in the X-linked immunoglobulin superfamily, member 1 (IGSF1) gene cause a novel, X-linked form of congenital central hypothyroidism in humans. In two mouse models of IGSF1-deficiency, we observed significant reductions in pituitary Trhr1, resulting in impaired TRH stimulation of TSH secretion. One possible mechanism for reduced Trhr1 is that thyrotropes of knockout mice are more sensitive to TH negative feedback. TH sensitivity is regulated by hormone transport, metabolism, and/or receptor activation. A potential role for IGSF1 in transport derives from our observation that IGSF1 physically interacts with a TH transporter, monocarboxylate transporter 8 (MCT8). However, we did not observe any change in MCT8-mediated transport of THs in the presence of IGSF1. We therefore hypothesize that IGSF1 functionally interacts with a structurally-related transporter in thyrotropes. We are presently taking two approaches to identify the relevant transporter. First, we are defining the domains mediating the MCT8:IGSF1 interaction. By mapping the relevant domain in MCT8, we can screen transporters with sequence homology. Second, our recent RNAseq analysis of murine pituitary revealed high levels of L-type amino acid transporter 2 (LAT2), identified as a TH transporter in vitro. We are currently examining the functional and physical interaction between IGSF1 and LAT2.

Funding Source: Supported by CRRD (EB), NSERC (EB), CIHR (DJB)

POSTER 2

PRENATAL EXPOSURE TO 1,2-CYCLOHEXANE DICARBOXYLIC ACID DIISONONYL ESTER (DINCH) ON OFFSPRING LEYDIG CELLS AND TESTOSTERONE PRODUCTION

Enrico Campioli[1,2], Matthew Lau[1,4], Sunghoon Lee[1,3], Lucas Marques[1,3] and Vassilios Papadopoulos[1,2,3,4,5]

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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 that come into contact with food. Although DINCH received final approval from the European Food Safety Authority in 2006, there is limited knowledge about its potential endocrine-disrupting properties. Bisphenol A, a chemical used as an intermediate in polycarbonate plastic and epoxy resin synthesis, and phthalate plasticizers, have been shown to be associated with the development of endocrine and reproductive diseases and different types of cancer. Preliminary studies in our laboratory showed altered gene profile in the testis of PND 3 and 6 pups that had been exposed in utero to DINCH. Moreover, DINCH exposure resulted in a non-monotonic reduction of serum testosterone levels and seminal vesicle weight in the PND 60 progeny. The purpose of the present work was to assess whether *in utero* exposure to 1, 10 and 100 mg DINCH/kg/day from gestational day 14 until birth would affect the progeny testis function.

In utero exposure to DINCH did not affect body weight and anogenital distance of the male offspring at PND 3 and 200, but it affected the anogenital distance at PND 60. Gene markers of somatic and germ cell function in the testis, including steroid production and androgenic activity, were analyzed. PND 3 pups exhibited a modification in *Nes* and *Cyp11a1*, which are highly expressed in Leydig cells. Additional genes were modified in PND 60 animals: *Star*, *Tspo*, *Cyp11a1*, *Ar*, and *Plzf*. At PND 200 only *Cyp11a1* and *Pdgfra* were significantly modified. Testosterone production was reduced significantly at both PND 60 and PND 200. Culture of PND 3 testes with DINCH did not affect testosterone production and thus had no effect on fetal Leydig cells. Seminal vesicle weights at PND 60 and 200 were negatively correlated to in utero DINCH dose. Interestingly we observed the random appearance in PND 200 animals of small and liquid testis containing degenerating tubules.

Taken together, these results suggest that DINCH might have a direct effect on Leydig cell function, causing a premature aging of the testis. Those effects are likely attenuated with the physiological aging of the animal.

Funding Sources: Supported by CIHR grant FRN-148688 and a CRC).

POSTER 3

ACTIVINS STIMULATE HUMAN FOLLICLE-STIMULATING HORMONE ß (FSHB) EXPRESSION VIA A FOXL2-DEPENDENT MECHANISM IN PITUITARIES OF TRANSGENIC MICE

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Follicle-stimulating hormone (FSH) synthesis by pituitary gonadotrope cells is stimulated by hypothalamic GnRH and intra-pituitary activins. Activins bind complexes of type I/type II receptor serine/threonine kinases, which phosphorylate SMAD proteins. SMAD complexes then accumulate in the nucleus where they bind the FSHß subunit (*Fshb)* promoter in combination with forkhead box L2 (FOXL2). Deletion of *Smad4* and/or *Foxl2* genes in gonadotrope cells causes profound FSH deficiency in mice. However, the mechanisms of activin-regulated human *FSHB* expression are relatively unknown. FOXL2 is expressed in human gonadotropes and can bind to the proximal human *FSHB* promoter *in vitro*. We examine the role of FOXL2 in activin regulation of human *FSHB* expression. Mice harboring a 10 kb human *FSHB* transgene (hereafter hFSHB) express the corresponding mRNA and protein specifically in gonadotrope cells. Murine *Fshb* mRNA expression was stimulated by exogenous activins in cultures from male or female mice. Basal *Fshb* expression was blocked by follistatin-288 or the type I receptor inhibitor SB431542. Similarly, in the female pituitary cultures, activins stimulated and the inhibitors attenuated h*FSHB* mRNA levels. In contrast, h*FSHB* mRNA expression was only modestly regulated by activins or the inhibitors in male cultures. Further, we crossed h*FSHB* transgenic mice with animals carrying floxed alleles for *Foxl2*. FOXL2 ablation *in vitro* impaired basal and activin-stimulated murine *Fshb* and human *FSHB* mRNA levels. Thus, the human *FSHB* gene is activin responsive and is dependent on FOXL2 for its expression. These results suggest that mechanisms of *Fshb/FSHB* regulation by activins and FOXL2 are conserved across species.

Funding sources: Supported by CIHR MOP-133394 to DJB and Ferring Inc. PDF Scholarship to LO

POSTER 4

THYROTROPIN-RELEASING HORMONE MAY POSITIVELY REGULATE RELAXIN-3 MRNA EXPRESSION IN MURINE PITUITARY

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Relaxin-3 (RLN3) is a member of the insulin superfamily that has previously been implicated in regulation of the hypothalamic-pituitary-gonadal (HPG) and hypothalamic-pituitary-adrenal (HPA) axes. Following the finding that *Rln3* expression is increased by 100-fold in the pituitaries of male mice with diet-induced hypothyroidism, we hypothesized that *Rln3* may also play a role in the hypothalamic-pituitary-thyroid (HPT) axis. The findings in this paper show that there is an increase in pituitary *Rln3* expression in male mice following once daily injections of TRH for 3 days. However, this effect could not be replicated in a follow up experiment featuring once daily injections of TRH for 3 and 5 days. No stimulatory effects were observed upon treating immortalized pituitary cell line with TRH *in vitro*. The results of the current study are inconclusive; however, RLN3 clearly has an important role in the hypothyroid murine pituitary as evidenced by its a robust increase in expression. This paper proposes a negative feedback mechanism in which RLN3 exerts a negative feedback effect in the paraventricular nucleus (PVN) of the hypothalamus to inhibit *Trh* expression. Further studies should be conducted to elucidate the involvement of RLN3 in the HPT axis and test the validity of the proposed novel negative feedback loop between the pituitary and the PVN to broaden our current understanding of the HPT axis.

Funding Sources: Supported by CIHR.

POSTER 5

$G\alpha_{q/11}$ AND $G\alpha_s$ PLAY IMPORTANT AND DISTINCT ROLES IN GONADOTROPE CELLS *IN VIVO*

Chirine Toufaily[1*], Han K. Kim[2*], Rona S. Carroll[2], Uli Boehm[3], Lee S. Weinstein[4], Stefan Offermanns[5], Daniel J. Bernard[1^], and Ursula B. Kaiser[2^]

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GnRH acts via the GnRH receptor (GnRHR) to regulate LH and FSH biosynthesis and secretion by pituitary gonadotrope cells. Classically, the GnRHR was thought to signal uniquely via the G proteins, $G\alpha_q$ and $G\alpha_{11}$ ($G_{q/11}$), to activate a PLC-DAG-PKC-MAPK cascade to regulate gonadotropin synthesis, specifically LH. GnRHR can also couple to $G\alpha_s$ (G_s), however, its role is still unclear. To understand the relative roles of different G proteins in gonadotropin production *in vivo*, we generated mice lacking $G_{q/11}$ or G_s specifically in gonadotropes using a Cre-lox approach. To produce $G_{q/11}$ conditional knockouts (KOs), we crossed *Gnaq*$^{fl/fl}$; *Gna11*$^{-/-}$ with GRIC mice, which express Cre recombinase from the endogenous *Gnrhr* locus. Both male and female $G_{q/11}$ KOs failed to go through pubertal maturation. We similarly produced gonadotrope-specific G_s KO mice by crossing *Gnas*$^{fl/fl}$ and GRIC mice. G_s KO females were fertile, producing normal sized litters at normal frequencies. Adult G_s KO males exhibited significant reductions in testis mass and pituitary expression of the FSH subunit genes (*Fshb* and *Cga*) as well as of *Gnrhr* relative to controls. In contrast, these males did not show differences in *Lhb* expression or in circulating levels of FSH or LH. These data so far suggest that G_s may play a greater role in FSH than in LH synthesis and that both gonadotropins may depend on intact $G_{q/11}$-dependent signaling. As these G proteins mediate the actions of multiple ligands, the relative contributions to the observed phenotypes of altered signaling by the GnRHR relative to other GPCRs in gonadotropes remain to be determined.

Funding Sources: Supported by NIH and CIHR

POSTER 6

ASSESSING THE EFFECT OF BODY CONDITION LOSS ON HEPATIC AND OVARIAN TISSUE FUNCTION DURING THE TRANSITION PERIOD IN DAIRY COWS

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The transition period involves stressful adaptation of energy metabolism in dairy cows. The inability to meet the energy requirements leads to a state of negative energy balance, often accompanied by excessive lipid mobilization. Body condition scoring (BCS) provides a visual appraisal of lipid mobilization. Our aim was to investigate the effect of body condition loss on hepatic and ovarian functions in dairy cows during the transition period until onset of breeding. Holstein cows were monitored for changes in BCS from 4 weeks pre-calving until 8 weeks post-calving. They were retrospectively grouped by changes in BCS with Group 1 (moderate; n=9) cows that lost < 0.75 BCS and Group 2 (severe; n=8) cows that lost ≥ 0.75 BCS. We collected liver biopsies at -3 weeks, near day of calving and +7 weeks. The last liver biopsy was accompanied by collection of granulosa cells (GCs) from the dominant follicle. Ovarian function was evaluated through mRNA abundance of genes required for follicular development (*FSHR* and *LRP8*) in GCs, which remained constant among groups. In hepatocytes, *CYP7A1* transcripts gradually increased ($P<0.05$) in Group 2 from -3 weeks to +7 weeks, but remained constant in Group 1. Other transcripts involved in lipid metabolism (*LDLR* and *SCARB1)* remained unaltered between the groups. Considering our previous data that lactating cows have higher bile acids than heifers, we conclude that cows with severe BCS loss experienced an increase in bile acid synthesis through higher expression of *CYP7A1*. These increased bile acids may have negative effects on ovarian functions.

Funding: Supported by FQRNT

POSTER 7

THE ROLE OF X-CHROMOSOME ASYNAPSIS IN THE ELIMINATION OF MURINE OOCYTES

Brandon Vaz[1] & Teruko Taketo[1,2].

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Women's fertility is limited by the number and quality of oocytes in the ovarian reserve, which is largely established around birth. The oocyte reserve is finite because all oocyte precursors stop proliferation and enter meiosis in early fetal life; in addition, more than 70% of the initial oocyte population is eliminated before birth. Whether this major oocyte loss is necessary or preventable remains controversial. The oocyte goes through Meiotic Prophase I (MPI), wherein homologous chromosomes synapse and recombine, while this major oocyte loss occurs. Since an error in meiotic synapsis would result in aneuploidy, it has been hypothesized that oocytes with meiotic errors are eliminated by a surveillance mechanism. The aim of my study is to test this hypothesis in the XO and XY sex-reversed female mice, in which the single X chromosome is missing its pairing partner. We have previously reported that the X chromosome in 72% of XO and 90% of XY oocytes at the pachytene stage of MPI are positive for phosphorylated H2AX (γH2AX), a marker of unsynapsed chromatin domains associated with a transcriptional silencing process named MSUC (meiotic silencing of unsynapsed chromatin). It remains an enigma as to why some XO or XY oocytes are γH2AX-negative and escape elimination despite the single X chromosome. The first objective of my study is to clarify the association between the presence or absence of γH2AX domain in the oocyte and its survival. However, estimating oocyte populations in histological sections of postnatal ovaries is unreliable because of the diversity in oocyte size. Recently, I developed a novel computer-aided method to identify and count the oocytes in whole-mount neonatal ovaries. Using this method, we found that the oocyte reserves of both XO and XY ovaries are reduced by 80% compared to their XX controls by 4 days after birth. In parallel, the proportion of γH2AX-positive oocytes also diminished in both XY and XO ovaries, suggesting that the oocytes are indeed eliminated by MSUC. In the future, we will examine whether this elimination is through apoptosis, and if so, by which pathway.

Funding Sources: Supported by CIHR grant to Teruko Taketo and McGill Claude Gagnon Urology Studentship to Brandon Vaz.

POSTER 8

A SINGLE-STEP CHROMATOGRAPHIC METHOD FOR THE PURIFICATION OF HUMAN AND MOUSE RECOMBINANT BINDER OF SPERM (BSP) PROTEINS

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Binder of SPerm (BSP) protein represent a super-family exclusively expressed in the male reproductive tract and they play a role in fertilization. Recently, a human BSP homolog 1 (BSPH1) and two mouse BSP homologs (BSPH1 and BSPH2) have been identified. Since these proteins are produced in very minute quantities in these organisms, we expressed His-tagged recombinant proteins in *E.coli* and purified using His-bind columns. However, recombinant proteins purified on His-bind column were still impure and are not suitable for function analysis. In previous study, we have shown that BSP proteins interact with pseudo-choline groups such as Diethylaminoethyl (DEAE) by affinity interaction rather than ionic interactions. The aim of the current study was to develop a method to purify recombinant BSP proteins using DEAE-Sephadex. Mouse and the human recombinant BSP proteins were expressed in Origami B (DE3) pLysS. The recombinant proteins were then extracted from cells with B-Per bacterial protein extraction reagent containing 3 M urea and passed through DEAE-Sephadex column. The column was washed with 1 M NaCl in Tris-buffer and the bound BSP proteins were eluted with 8 M urea. The fractions eluted with urea were pooled, desalted and lyophilized. The recombinant proteins (mouse-BSPH1, mouse-BSPH2 and human-BSPH1) purified using this method were more than 95% pure as judged by SDS-PAGE and immunoblots using specific antibodies. In conclusion this simple and efficient novel method would be useful for further characterization and to clarify the role of BSP proteins in fertilization.

Funding Sources: Supported by CIHR and Maisonneuve-Rosemont Hospital Foundation

POSTER 9

CRITICAL ROLE OF PEROXIREDOXINS IN PROTECTING MOUSE SPERMATOZOA AGAINST OXIDATIVE STRESS

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Peroxiredoxins (PRDXs) are the main antioxidant defenses against oxidative stress in human spermatozoa. *Prdx6*$^{-/-}$ mouse spermatozoa have poor reproductive outcomes. Our aim was to elucidate the impact of the inhibition of 2-Cys PRDXs or the lack of PRDX6 on fertilizing competence of mouse spermatozoa.

C57Bl/6J wild type (WT) mice spermatozoa were incubated in BWW medium with MJ33 (PRDX6 PLA_2 activity inhibitor), or thiostrepton (TSP; 2-Cys PRDX peroxidase activity inhibitor) for 2h at 37°C. Sperm motility was measured by CASA. Embryo development, zona binding, sperm fusion were evaluated after IVF with mature oocytes. Sperm viability, lipid peroxidation, and mitochondrial superoxide ($O_2^{\bullet-}$) levels were determined by flow cytometry using Sytox Green, BODIPY C11 and MitoSOX Red, respectively. Acrosome reaction was assessed by FITC-PSA labeling.

Sperm motility, viability, zona binding, fusion, fertilization and blastocyst rates were lower in *Prdx6*$^{-/-}$ spermatozoa than in WT controls ($p<0.05$). MJ33 or TSP (10 or 20 µM) reduced these parameters in WT spermatozoa compared with controls ($p<0.05$). Levels of lipid peroxidation and of $O_2^{\bullet-}$ were higher in *Prdx6*$^{-/-}$ than in WT spermatozoa ($p<0.05$). MJ33 and TSP significantly increased levels of lipid peroxidation and $O_2^{\bullet-}$ in treated vs. non-treated WT spermatozoa. Acrosome reaction was lower in *Prdx6*$^{-/-}$ capacitated spermatozoa than WT controls.

In conclusion, the inhibition of PRDXs and/or the PRDX6 PLA_2 activity promotes an oxidative stress affecting viability, motility and the ability of mouse spermatozoa to fertilize oocytes. PRDXs particularly PRDX6 have a critical role in the protection of the mouse spermatozoon against oxidative stress.

Funding Sources: Funded by CIHR

POSTER 10

DISRUPTION OF THE PEROXIREDOXIN SYSTEM AFFECTS HUMAN SPERM VIABILITY AND DNA INTEGRITY

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Our laboratory is pioneer in characterizing the family of antioxidant called peroxiredoxins (PRDXs) and establishing their role in the antioxidant protection of human spermatozoa. Indeed, we found that spermatozoa from infertile men have inactive PRDXs. However, it is unknown whether human spermatozoa have an active PRDX re-activation system. Thus, we aimed to determine the impact of the inhibition of different players of the PRDX system on the reactive oxygen species (ROS) levels, viability and DNA oxidation in human spermatozoa.

Highly motile spermatozoa from healthy donors were incubated with inhibitors of the PRDX system and viability, ROS levels and DNA oxidation were assessed by flow cytometry using Calcein-AM, MitoSOX-Red ($O_2^{\bullet-}$), APF and H_2DCFDA ($HO^{\bullet}$, ONOO), and the anti-8-OHdG antibody, respectively.

We observed a significant decrease in viability in a dose dependence manner when incubated with inhibitors of the 2-Cys PRDXs (Conoidin A), calcium independent phospholipase A_2 (PRDX6-PLA_2) activity (MJ33) or the PRDX re-activation system (Auranofin, Ezatiostat, S-Hexylgluthatione and Ethacrynic acid) compared to controls ($p<0.05$). Inhibition of PRDX6-PLA_2 had the strongest detrimental effect on viability promoting a significant increase of $HO^{\bullet}$, $ONOO^-$, $O_2^{\bullet-}$ and DNA oxidation labeling compared to controls ($p<0.05$). Inhibition of glucose-6-Phosphate, isocitrate and malate dehydrogenases (DHEA, oxalomalic acid and 3-bromo-pyruvate, respectively), that supply NADPH for the 2-Cys PRDXs re-activation, decreased viability and increased $O_2^{\bullet-}$ levels in spermatozoa.

PRDXs, especially PRDX6-PLA_2 activity, are essential in maintaining viability and DNA integrity in spermatozoa. This study will help to design new diagnostic tools and personalized therapy to treat male infertility.

Funding Sources: Supported by CIHR and RI-MUHC fellowship to MCF.

POSTER 11

BINDER OF SPERM (BSP) PROTEIN CAN INTERFERE IN SPERM-EGG INTERACTION AND FERTILIZATION

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Epididymis possesses segment-specified secretions, which protect sperm and provide environment for sperm maturation. Binder of SPerm (BSP) protein is exclusively expressed in the human and murine epididymis. Recent studies show that BSP protein induces membrane modification events that occur during sperm capacitation. Upon capacitation, BSP protein migrates from the sperm surface to the equatorial segment, the site of sperm-egg fusion, suggesting a further role in sperm-egg interaction. Therefore, we examined the mouse recombinant BSP homolog 1 (rec-BSPH1) in sperm-egg interaction using *in vitro fertilization* (IVF) assay. Mouse rec-BSPH1 was produced in *E. coli* and purified on Ni-charged affinity chromatography. Oocytes were recovered from super-ovulated females. Zona-intact oocytes were co-incubated with different concentrations of rec-BSPH1 or control proteins and inseminated by capacitated sperm. After 20 h, fertilization rate was determined and analyzed. The study revealed that there was a dose-dependent inhibition (20-80%) of fertilization with oocytes pre-exposed to rec-BSPH1 compared to control (without protein). Furthermore, under the same conditions there was no inhibition of fertilization by control proteins such as ovalbumin and recombinant thioredoxin indicating that the inhibitory effect by rec-BSPH1 was specific. Mouse rec-BSPH1 showed inhibitory effect on fertilization, suggesting exogenous protein was capable of saturating specific sperm binding sites on the egg membrane and hindering sperm-egg interaction. Globally, there is an ongoing demand to develop a male contraceptive with fewer or no side effects. Notably, by understanding the precise role of BSP during sperm-egg interaction, it would be possible to target this protein for developing male contraceptive.

Funding Sources: CIHR and Maisonneuve-Rosemont Hospital Foundation

POSTER 12

IDENTIFICATION OF CLAUDIN SEQUENCE VARIANTS IN CHILDREN WITH CONGENITAL KIDNEY MALFORMATIONS

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To understand congenital kidney malformations, we need to define the molecular events that are critical for kidney formation. Kidney development begins with an aggregate of mesenchymal cells that epithelialize to form the nephric duct. This duct elongates caudally and an outgrowth known as the ureteric bud develops at the level of the hind limb. The ureteric bud gives rise to the collecting duct system and this process is dependent on reciprocal signaling between the adjacent mesenchymal cells and the ureteric bud epithelium. We focus on claudins, integral tight junction transmembrane proteins expressed during kidney development. Claudins regulate the intercellular barrier and pore properties of epithelia, and are required for the formation of tight junctions. Previously we showed that removal of a subset of claudins from tight junctions results in branching defects in the mouse embryonic kidney and disrupts nephric duct elongation in the chick embryo. Through *in situ* hybridization, we observed that *Claudin-1* and *-3* are expressed in the nephric duct in the chick embryo at Hamburger Hamilton stages 10 through 20. In the mouse embryo, we have shown that all Claudin family members are expressed in the kidney at embryonic day 13.5. We hypothesize that CLDN sequence variants will disrupt nephric duct elongation and/or ureteric bud branching resulting in congenital kidney malformations. We sequenced 24 claudins in 96 patients with renal malformations from the NIH-sponsored CKiD (Chronic Kidney Disease) study. We identified fifteen rare and five novel non-synonymous variants. The variants are now undergoing functional assessment.

Funding sources: Kidney Foundation of Canada

POSTER 13

IMPROVED PROTOCOL FOR ACTIVATION OF PORCINE OOCYTES

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Oocyte activation is important for production of embryos generated parthenogenetically, by nuclear transfer and sperm injection. During this event, Ca^{2+} oscillations followed by Zn^{2} exocytosis events lead to meiotic resumption. Our aim is to evaluate different agents to establish an improved protocol for activation of porcine oocytes. First, we assessed pronuclear (PN) rates after: A) 5 min of Ionomycin (Ion); B) Ion + CDK1 inhibitor); C) Ion + PKC activator); and D) Ion + CDK1i + PKCa (Combo). With the exception of group A, all groups were treated for 1, 2, 3 or 4h post Ion treatment. The 4h treatment showed higher PN rates, however, was lower for PKCa (18.7%) compared with CDK1i (41.7%) and Combo (58.8%). PN rates after treatment with Ion followed by a Zn^{2+} chelator (Ion + TPEN) was accessed for 5, 10, 15, 20, 25 or 30 min. PN rates were lower for TPEN 5 and 10 (<85%) compared with time points greater than 15 (>90%). Lastly, we evaluated embryo development and cell number with: I) Ion + TPEN 15min; II) Ion + TPEN 30min; III) Ion + TPEN 15min + Combo 4h; or IV) Ion + TPEN 30min + Combo 4h. Blastocyst rates were higher in group I (79.8%) compared with groups II, III and IV (<58%). Cell number in blastocysts was higher in groups I (63.6±7.3) and II (46.1±5.3) compared with III (39.6±4.6) and IV (40.2±6.1). Therefore, treatment with Ionomycin followed by TPEN for 15 minutes provides an effective protocol for porcine oocyte activation.

Funding Sources: Supported by the Natural Sciences and Engineering Research Council of Canada (NSERC).

POSTER 14

NAÏVE TO PRIME PLURIPOTENCY TRANSITION IS MEDIATED BY PRIMITIVE ECTODERM-LIKE CELLS

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Naïve and primed pluripotent stem cells in *in vitro* cultures are most analogous to embryonic day 4.5 (E4.5) inner cell mass epiblast cells and E6.5 anterior primitive streak cells respectively. However, pluripotent epiblast cells within peri-implanted E5.5 embryos remain enigmatic. Here we develop a 3D culturing system to differentiate naïve mESC into cysts resembling the E5.5 epiblast. We found that within 72h in 2i-Lif free conditions ESC self-organize and polarize into monolayer cysts with luminal apical domain. RT-qPCR analysis showed downregulation of *Rex1* and *Fgf4* by 72h indicating exit of naïve pluripotency but no upregulation of Nodal/Activin pathway associated genes, which are upregulated in *in vivo* E5.5 epiblast and primed EpiSCs. Wt ESC colonies treated with either Mek or FGFR inhibitors and *Fgf4* KO ESC colonies were unable to exit naïve pluripotency and developed into amorphous clusters with lower sphericity and roundness. Supplementation of FGF4 and Heparin to *Fgf4* KO ESC colonies was sufficient to restore exit of naive pluripotency and cyst formation. These results demonstrate that naïve exit is dependent on FGF/ERK pathway stimulation, and 3D culturing ESC in the absence of Nodal/Activin signaling causes ESC to exit naïve pluripotency but not yet enter primed pluripotency.

Funding Sources: Supported by NSERC, CHIR, McGill Faculty of Medicine, Center for Research in Reproduction and Development

POSTER 15

NON-CANONICAL FUNCTIONS OF E-CADHERIN IN THE PREIMPLANTATION MOUSE EMBRYO

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Preimplantation mammalian development consists of a series of key events; polarization, asymmetric division and differential gene expression, leading to the formation of a blastocyst. Polarization is the first event of divergent cell lineage specification initiated in an 8-cell stage embryo. Non-polarized 8-cell blastomeres undergo the formation of an apical domain on non-contact surfaces. Polarized 8-cell blastomeres can divide asymmetrically to generate the first two cell types, polar cells inheriting the apical domain and apolar cells not inheriting it. The polar cells are allocated on the surface, while the apolar cells internalize to take an inner position resulting in the generation of the trophectoderm (TE), which gives rise to the placenta and the inner cell mass (ICM) which further differentiates into the epiblast and primitive endoderm that give rise to the fetus and yolk sac, respectively.

E-cadherin is known for their function in cell-cell adhesion through their extracellular cadherin domain and enriched at cell-cell contact. Interestingly, we found that E-cadherin was also enriched on non-contact surfaces during the initial polarization phase at the 8-cell stage. Additionally, when we analyzed maternal zygotic *E-cadherin* mutant embryos, in which E-cadherin was completely eliminated, we found precocious polarization at the 4-cell stage. These results suggest that E-cadherin has an adhesion-independent role on non-contact surface to negatively regulate polarization in the early 8-cell embryo.

Funding Source: McGill University Faculty of Medicine

POSTER 16

THE ROLE OF CLAUDINS DURING BRANCHING MORPHOGENESIS IN CHICK LUNG

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Claudins are a family of proteins located in the tight junctions of epithelial cells. More than twenty members are found in vertebrates and each has unique permeability characteristics, which affect the barrier properties of the tight junction. Claudins can be observed in numerous tissues of the human body where they are involved in paracellular transport. They are also widely expressed throughout development, including during branching morphogenesis of several organs. Previous data from my lab have shown that removing claudins from the tight junctions of embryonic mouse kidneys decreased branching morphogenesis. From these results, we hypothesize that claudins have a common role in branching morphogenesis of the kidneys and the lungs. To study claudin function in lung morphogenesis, I first characterized claudin expression in the embryonic lung. By whole mount *in situ* hybridization, I found that *Cldn1*, *Cldn3* and *Cldn10* are expressed in the bronchi and the buds of the chick developing lung from E4 to E6. I also found that *Cldn4* and *Cldn5* are faintly expressed in the buds of embryonic chick lungs at E5. Additionally, I optimized conditions to culture chick lung explants on a membrane for up to 72 hours. Next, I want to determine if the removal of specific claudins affects branching morphogenesis in the lungs. To do that, I will treat chick lung explants with a truncated nontoxic version of the *Clostridium perfringens* enterotoxin that removes a subset of claudins including Claudin-3 and -4 which are expressed in the embryonic lungs.

Funding Sources: Supported by NSERC.

POSTER 17

YEAST-DERIVED MICROBIAL PROTEIN SUPPLEMENTATION TO TRANSITION DAIRY COWS ALTERS HEPATIC GLUCONEOGENIC GENE EXPRESSION

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In transitioning dairy cows, drastic metabolic changes occur in response to calving and lactation. These metabolic changes often result in negative energy balance (NEB), which can lead to infertility and disease susceptibility. The aim of this project was to evaluate or determine the effect of a yeast-derived microbial protein feed supplement on hepatic glucose production and, thereby promote fertility. For this, we supplemented transition dairy cows with or without a yeast-derived microbial protein (YMP; Bélisle Solution Nutrition Inc.) as gluconeogenic precursors. Treated cows (n=6) were supplemented with 50g of YMP daily for 3 weeks before calving and 200g daily for 4 weeks after calving. Control cows (n=13) received no supplementation. Liver biopsies were performed at 3 weeks pre-partum, the week of calving and 7 weeks post-partum. Granulosa cells and follicular fluid from the dominant follicle of a synchronized wave were collected at 7 weeks post-partum. Blood samples were collected at weeks -1, 0 and +1 relative to each liver biopsy. Quantitative PCR analysis revealed that genes associated with hepatic gluconeogenesis were significantly higher (Glucose-6-Phosphatase; *G6PC*) or had a tendency to be higher (Phosphoenolpyruvate-carboxykinase-1; *PCK1*) in YMP-supplemented cows across the study period compared to control cows. Analysis of blood glucose levels revealed a significantly lower level at 1 week post-calving compared to 3 weeks prepartum in control cows that was not observed in supplemented cows. Based on gene expression data and blood biochemistry, our preliminary results suggest that YMP supplementation increases gluconeogenc activity in transition dairy cows, thereby allowing for more consistent blood glucose levels.

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POSTER 18

TARGETING CLAUDINS TO MODIFY TIGHT JUNCTION BARRIER PROPERTIES

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Claudins are essential for tight junction (TJ) formation and function. The combination of claudins within an epithelial cell layer determines its paracellular barrier properties. Nutraceuticals like quercetin can modify claudin expression, while molecules such as the non-toxic C-terminal domain of *C. perfringens* enterotoxin (C-CPE) can bind to and remove a subset of claudins from TJs. To alter the specificity of C-CPE, we replaced its claudin binding domain with the amino acid sequence of the second extracellular loop of individual claudins. Currently, we are testing the ability of these substances to transiently modify the claudins and TJ barriers. We showed that all three approaches modify claudin expression, localization or postranslational modifications affecting the TJ barrier properties in different ways. Our goal is to develop tools that can be used to modify the TJ composition and barrier properties to extend the use of these reagents to treat disease and improve health.

Funding Sources: CIHR

POSTER 19

BINUCLEATION FAILS TO ACTIVATE A TETRAPLOIDY CHECKPOINT AND INSTEAD CAUSES SEGREGATION ERRORS IN THE PREIMPLANTATION EMBRYO.

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Binucleation is commonly observed in human embryos, although its consequences to development are poorly understood. The aim of this study is to understand the impact of binucleation in the mammalian preimplantation embryo. Binucleation was induced in embryos at the 4-cell stage and embryo development, cell cycle progression and chromosome segregation errors analysis were performed. For fixed imaging, the embryos were labeled for DNA, plasma membrane, kinetochores and centromeres. For live cell imaging, we used markers of chromosomes, cell cycle, spindle poles and microtubules. Live and fixed images were acquired using either confocal or structured illumination microscopy. The number of cells within binucleated embryos increased similarly to diploid cells, indicating that binucleation does not prevent embryonic cell divisions. Cells within binucleated embryos possessed approximately double the amount of kinetochores and centromeres as the control diploid group, confirming that binucleation leads to tetraploidy. Using live imaging of PCNA:EGFP we observed that the cell cycle occurred in binucleated embryos with normal temporal dynamics. However, we observed that embryos that had been binucleated possessed substantially greater numbers of micronuclei than the control diploid group. Consistent with this, with live cell imaging, we observed higher rates of abnormal divisions as compared to the control group, including lagging chromosomes, chromosome misalignment, and chromosome bridges. Live imaging of spindle poles and microtubules revealed altered microtubule dynamics during mitosis. We conclude that embryos lack a tetraploidy checkpoint, and we propose that tetraploidy may be a previously unappreciated stepping stone to the generation of aneuploid cells in embryos.

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POSTER 20

DEFICIENCY OF BSPH2, AN EPIDIDYMAL PROTEIN, CAUSE NO EFFECT ON FERTILITY IN MICE

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Prior to fertilizing an oocyte, sperm are subjected to numerous biochemical and physiological changes, which are induced by abundant secreted molecules in the male and female reproductive system. A family of protein named Binder of SPerm (BSP) was first identified in our laboratory in bovine seminal plasma. Previous studies have indicated that the binding of these proteins to sperm membranes via choline phospholipids mediate important changes in the lipid composition, which there by promote sperm capacitation leading to acrosome reaction. In the past few years, BSP-homologous genes have been identified in both human (*BSPH1*) and mouse (*Bsph1* and *Bsph2*) epididymis. Mouse BSPH1 has been shown to promote sperm capacitation but not BSPH2, suggesting that the latter protein might play a different role in sperm functions. The aim of the current study was to establish the exact role of mouse *Bsph2 in vivo.* Mice lacking *Bsph2* expression were generated using the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR-associated 9 (Cas9) technology. Following *in vivo* studies, no obvious phenotype was observed after fertility analysis for $Bsph2^{-/-}$ mice. No differences were noticed in the average pup weight and the average litter size. Normal testis weight and morphology were observed in $Bsph2^{+/-}$ *and* $Bsph2^{-/-}$ compared to wild type. Quantitative PCR analyses revealed that *Bsph1* mRNA expression in mutant significantly increased compared to wild type indicating that loss of *Bsph2* could be compensated by *Bsph1* upregulation, which might be the reason of observing any phenotype. Taken together, this study revealed that *Bsph2* could be a complementary protein for *Bsph1*.

Funding Sources: CIHR

POSTER 21

CLAUDINS REGULATE CELL SHAPE AND LOCALIZATION OF SIGNALING PROTEINS AT THE APICAL CELL SURFACE DURING NEURAL TUBE CLOSURE

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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, planar cell polarity and Par polarity 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: FRQ-S and NSERC

POSTER 22

SPINDLE ASSEMBLY CHECKPOINT INSUFFICIENCY CAUSES CHROMOSOME SEGREGATION ERRORS IN MOUSE PREIMPLANTATION EMBRYOS

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Chromosome segregation errors during early embryonic divisions result in embryos comprising a mixture of euploid and aneuploid cells. While mosaic aneuploidy is observed commonly in human embryos, how and why it arises remains elusive. In somatic cells, the spindle assembly checkpoint (SAC) is the major cellular safeguard against aneuploidy, delaying the onset of anaphase until all chromosomes have been correctly attached to spindle microtubules. Here we examine the presence, strength and sensitivity of the SAC in preimplantation embryos. We performed fast-acquisition live imaging of H2B:RFP-expressing embryos and found misaligned pre-anaphase chromosomes in ~5% of divisions, suggesting that SAC function is weak in early mouse embryos. To ascertain whether a SAC operates in preimplantation development, embryos were treated with SAC-specific inhibitor *AZ3146*. Immunofluorescence and live imaging experiments show SAC inhibition results in premature anaphase with increased chromosome misalignment and chromosome segregation errors (p=0.002). We next probed SAC strength by exposing embryos for 16h to different Nocodazole concentrations. Embryos only arrested in mitosis at the highest concentrations tested, despite significant spindle damage and increased segregation errors at lower concentrations. Furthermore, live imaging of embryos in low doses of Nocodazole (10nM), show no anaphase delay despite an increased rate of chromosome mis-segregation, suggesting that a failure to activate SAC signaling in response to spindle damage results in chromosome segregation errors. Taken together, our results demonstrate that in embryos the SAC acts to limit chromosome mis-segregation, but insufficiently sensitive or robust to prevent all errors. These findings provide a potential mechanistic explanation for mosaicism.

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POSTER 23

EFFECT OF THE INJECTION TIME AND INHIBITION OF DNA REPAIR ON GENOME EDITING EFFICIENCY IN PORCINE EMBRYOS PRODUCED BY ACTIVATION, IVF, ICSI AND SCNT

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The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR-associated nuclease (Cas) is a powerful technology that is being adapted for genome manipulation in different cell types and species. However, as a new technology, it still requires improvements. There is evidence that the cell cycle and DNA repair machinery can affect the genome-editing efficiency. Thus, we hypothesized that the zygote cell cycle stage when the CRISPR/Cas system is injected and the inhibition of homology directed repair (HDR) pathway can improve the efficiency of genome-editing in pig embryos. Porcine zygotes produced by parthenogenetic activation (PA), in vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI) or somatic cell nuclear transfer (SCNT), were injected with the CRISPR/Cas system before/early S-phase (between 0-5h after fertilization/activation) or after/late S-phase (between 14-16h after fertilization/activation), and without or with an HDR inhibitor until 48 h, when embryo cleavage was assessed. The method of embryo production did not significantly affect genome-editing efficiency in developing embryos, SCNT (85.7%), PA (83.3%), ICSI (72.7%), IVF (60.0%), average of all methods (76.5%). Zygotes injected before/early S-phase had a lower proportion of genome-edited embryos (71.4%) compared with those injected after/late S-phase (95.5%). Inhibition of the HDR pathway increased the proportion of genome-edited embryos injected before/early S-phase (87.0% vs 77.1%). Our findings show that the CRISPR/Cas system is highly efficient in creating genome-edited embryos by injecting pig zygotes produced by different technologies. Injection of zygotes after/during late S-phase and inhibition of HDR pathway in zygotes injected before/early S-phase can further increase genome-editing efficiency in porcine embryos.

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POSTER 24

GENERATION OF LIVER SPECIFIC KNOCKOUT OF TMED2 TO INVESTIGATE ITS REQUIREMENT DURING EMBRYONIC LIVER DEVELOPMENT

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TMED2 is a member of the transmembrane emp24 domain-containing (TMED) family of cargo receptors involved in the secretory pathway and expressed in the developing mouse liver. Mutant mouse embryos lacking TMED2 protein die by embryonic day (E) 11.5, precluding analysis of its role in liver development. In contrast, though *Tmed2*$^{+/-}$ mice survive until adulthood, a significant number develop non-alcoholic fatty liver disease indicating a role in liver function. We hypothesize that TMED2 is required for the proper development of the liver. TMED2 protein levels are significantly decreased in livers of E17.5 *Tmed2*$^{+/-}$ embryos. To determine if decreased protein affects liver cell fate, we compared the profile of hepatocytes, megakaryocytes, granulocytes and erythrocytes in H&E-stained liver sections of *Tmed2*$^{+/-}$ E17.5 embryos: no significant difference in proportions of these cell types was observed in E17.5 males suggesting that cell differentiation is unchanged. Concomitantly, we will flank exon 2 of *Tmed2* with loxP sites by CRISPR/Cas9-based homology directed repair. These conditional KO mice will then be mated with albumin promoter-driven cre-recombinase transgenic mice to remove *Tmed2* liver-specifically. Guide RNAs (gRNA) were designed and synthesized, two single-stranded DNA oligonucleotides and a double-stranded DNA gene fragment containing loxP sites (as DNA repair templates) were designed and ordered. Cas9 mRNA and gRNAs were micro-injected with either DNA oligos or gene fragment into mice blastocysts and transferred to pseudo-pregnant females. Of 45 born mice, 5 were positive for intron 1 loxP insertion and 3 were positive for intron 2 loxP insertion, however none were positive for both.

Funding Source: Supported by NSERC.

POSTER 25

PAX2 INDICATES TWO DEVELOPMENTALLY DISTINCT EPITHELIAL CELL POPULATIONS IN THE MOUSE OVIDUCT

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The Müllerian duct gives rise to organs of the vertebrate female reproductive tract in late embryogenesis, forming the oviduct from its most anterior region. The oviduct is a hollow epithelial tube that links ovary to uterus and serves in oocyte fertilization and embryonic transport during preimplantation. Early in the postnatal period, the complex oviduct develops from the simple Müllerian duct through coiling and formation of three morphologically distinct regions along its distal to proximal axis. Each region is distinguished by the ratio of ciliated to secretory cell types and extent of longitudinal folding in the luminal epithelium. To understand oviduct development, we used a PAX2-GFP BAC transgenic mouse model to visualize luminal epithelial cells of the Müllerian duct and oviduct. PAX8 marks oviduct secretory cells; its expression is lost during ciliogenesis. Given the functional redundancy of PAX2 and PAX8 elsewhere in the urogenital system, we expected that PAX2 might also mark secretory cells. Interestingly, we observed an absence of PAX2-GFP-positive cells in the distal tip of the Müllerian duct as early as E13.5, maintained through embryonic and postnatal development and adulthood, while PAX8 expression extended throughout the luminal epithelium of the Müllerian duct and neonatal oviduct until the emergence of multi-ciliated cells. Lineage tracing with a PAX8-Cre mouse line carrying the ROSA-LSL-tdTomato reporter suggest that PAX2-GFP-negative cells in the distal tip have higher *Pax8* expression. Our results indicate that complementary expression of PAX2 and PAX8 distinguishes two developmentally distinct epithelial cell populations in the distal and proximal mouse oviduct.

Funding Sources: CCSRI

POSTER 26

A NOVEL MOUSE MODEL FOR *Snap29,* A GENE DELETED IN THE 22q11.2 DELETION SYNDROME

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Introduction. 22q11.2 deletion syndrome (22q11.2DS) is a contiguous gene syndrome with a 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,* a gene that has been shown to contribute to the developmental syndrome, CEDNIK. We postulate that deletion of *SNAP29* contributes to the phenotypic spectrum of abnormalities found in a subset of 22q11.2DS patients. Our aims are to characterize expression of this gene during development and to generate a mouse model to determine where and when the gene is required for normal development

Methods. We used *in situ* hybridization to characterize expression of *Snap29* throughout mouse development. Mutant mouse lines with deletion in *Snap29* was generated with CRISPR/Cas9 technology. Homozygous mutant embryos and mice were analyzed using standard histological and molecular methods.

Results. The gene was expressed in embryonic precursors of organs that are affected in patients with 22q11.2DS and CEDNIK. In addition, expression was found in some additional sites not known to be affected in these patients. Our novel mouse knockout model for *Snap29* survived and show skin, brain and motor abnormalities. Currently we are investigating skin and brain defects seen in homozygous mice.

Conclusion. Our results indicate that deletion of *SNAP29* may contribute to subset of abnormalities in 22q11.2DS patients. The gene is deleted in 90% of patients and current mouse models for 22q11.2DS do not include *SNAP29*. Mouse model of *Snap29* will shed insight on the etiology of a subset of abnormalities found in CEDNIK and 22q11.2DS patients.

Keywords: 22q11.2 deletion syndrome, . CEDNIK, *SNAP29*

POSTER 27

EXTRACELLULAR MATRIX (ECM) ANOMALIES ARE ASSOCIATED WITH BLADDER DIVERTICULUM (BD) AND VESICOURETERAL REFLUX (VUR)

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VUR is the retrograde flow of urine from bladder toward kidneys due to developmental defects in the junction of ureter with bladder—the ureterovesical junction (UVJ). BD is the pouch formation within the bladder wall due to developmental defects in its musculature. Biomechanical properties of the ECM facilitate UVJ's closure during voiding and prevent bladder rupture during urine collection. Indeed, ECM syndromes such as Cutis Laxa, Marfan, EDS and Williams are characterized by the presence of VUR and BD. We use *Tnxb*$^{+/-}$ and *Tnxb*$^{-/-}$ mice on the C57Bl/6J background, which are a model of EDS, to investigate the effect of developmental defects in the ECM on the urinary tract. We developed a bladder compliance assay to evaluate risk of rupture and/or formation of BD in the newborn stage (P1). The bladders of *Tnxb*$^{+/-}$ and *Tnxb*$^{-/-}$ mice rupture at lower filling pressures compared to WT controls. Histological analysis of the bladder and ureter at P1 shows that the *Tnxb*$^{+/-}$ and *Tnxb*$^{-/-}$ mice has significantly less collagen, causing a reduction in tensile strength in the bladder leading to its rupture. We have sequenced *TNXB* in a cohort of children with VUR and EDS, and have identified a number of putative disease causing variants. The results suggest that the ECM is critical for the proper functioning of the UVJ and the bladder.

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POSTER 28

GENETIC INVESTIGATION OF RECURRENT HYDATIDIFORM MOLES AND REPRODUCTIVE LOSS

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Hydatidiform mole (HM) is an abnormal human pregnancy with excessive trophoblastic proliferation and abnormal embryonic development. HM can be sporadic or recurrent and can have different genetic makeups (diploid biparental, diploid androgenetic, triploid dispermic). Until now, there are no genes reported to be responsible for recurrent diploid androgenetic HM, which contain DNA from only the paternal genome and do not contain maternal DNA. A two-stage study was designed to identify new genes involved in this disease. First, Whole Exome Sequencing was performed in patients with at least two HMs. Candidate genes were selected and then screened in more patients with milder phenotypes by targeted-sequencing. With this approach, we identified three genes with recessive mutations and known to be involved in the same pathway. Patients with mutations in these genes have recurrent androgenetic HM. Recessive mutations in two of these genes were reported independently to cause infertility in mice and all genes were reported to affect the DNA double-strand break (DSB) formation and repair during meiosis. I am currently implementing an assay using ImageStream Flow Cytometer to measure the number of phosphorylated histone H2AX foci, a well-known marker for DSB, in cells of patients and controls after exposure to DSB-inducing agents. We are also studying an existing knockout mouse model for one of these genes to investigate how and when the maternal chromosomes are lost after fertilization and explain the mechanism of androgenesis. Our work will unravel for the first time the underlying molecular mechanism leading to androgenetic HMs.

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POSTER 29

TEMPORARY LOSS OF DNMT1 IN MOUSE ES CELLS LEADS TO ADAPTATIONS IN THE HISTONE MODIFICATION LANDSCAPE AND IN THE TRANSCRIPTOME

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Introduction: In early embryos, a major reprogramming wave resets genome-wide DNA methylation profiles. Throughout this wave, DNA methylation patterns, acquired in a parent-of-origin-manner on differentially methylated regions (DMRs), must escape this reprogrammation and sustain precise DNA methylation profiles through continuous DNMT1 (DNA methyltransferase 1) activity. Using an embryonic stem (ES) cell model with inducible *Dnmt1* repression (*Dnmt1*$^{tet/tet}$), we recently showed that a temporary lack of *Dnmt1* triggers the inherited loss of DMRs and DMR-like DNA methylation profiles. How inherited DNA methylation dysregulation emerges on specific loci following an embryonic loss of DNA methylation maintenance and how it can lead to severe growth abnormalities remains poorly understood.

Objective: Here, we investigate how the transient lack of *Dnmt1* influences the epigenetic landscape and global gene expression.

Results: Our RNA-seq experiment highlighted two noteworthy gene clusters; one set of genes that become strongly activated and one, strongly inactivated. We then explored by ChIP-Seq if the transient lack of DNMT1 protein prompts rearrangements in the histone landscape (H3K4me3, H3K27me3, H3K27ac) associated to these two gene clusters. For particular genes, we believe that alterations in the histone landscape impede the recruitment of DNMTs, as for others, the perturbations occur with the absence of neighbouring inherited loss of DNA methylation or histone marks.

Conclusion: The present study highlights new perspectives on how alterations in DNMT1-dependent methylation maintenance can alter DNA methylation profiles, histone modification cross-talk and gene expression as well as explain inherited epigenetic dysregulation events that occur in abnormal cells and during early embryo development.

Funding Sources: Supported by NSERC.

POSTER 30

DNA METHYLATION PERTURBATIONS AT MID-DEVELOPMENT IN MOUSE BRAIN AND PLACENTA AS A CONSEQUENCE OF EARLY EMBRYONIC INDUCTION OF FETAL ALCOHOL SPECTRUM DISORDER

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Prenatal alcohol exposure (PAE) is known to altered epigenetic profiles in cells during brain development and be part of the molecular basis underpinning Fetal Alcohol Spectrum Disorders (FASD) etiology. However, the consequences of a PAE during very early embryonic life on the future epigenetic landscape of embryonic and extraembryonic tissues remain unknown. **Our research hypothesis is that a PAE during pre-implantation will initiate DNA methylation dysregulation that will later be observable in the developing conceptus**. We believe that these original epigenetic alterations will be perpetuated and amplified in the developing brain as well as in the placental tissue. To test this, we instigated FASD in mouse 8-cell embryos by injecting ethanol at 2.5 days of pregnancy (E2.5). We collected FASD (ethanol) and control (saline) E10.5 embryos and placentas. We established genome-wide quantitative DNA methylation profiles of forebrains and placentas by Reduced Representation Bisulfite Sequencing. Bioinformatic analyses of FASD samples (n=12) vs controls samples (n=8) revealed 686 and 2942 differentially methylated tiles (DMTs) in forebrain and placenta samples respectively. Interestingly, we also uncovered 21 specific regions abnormally methylated in both FASD forebrain and placenta samples. Our study establishes for the first time that early embryonic PAE can cause epigenetic dysregulations that leads to permanent alteration in the future epigenetic program of brain and placenta cells. Altogether, 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.

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POSTER 31

DETERMINING THE IMPACT OF A HIGH-FAT DIET IN OFFSPRING SIRED BY A TRANSGENIC MOUSE MODEL WITH ENHANCED EPIGENOMIC SENSITIVITY

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Within the last decades, an upward trend in complex diseases rates has been observed worldwide. It is well known that genetic and lifestyle factors can lead to the development of obesity in adulthood. Overweight fathers are more likely to have overweight children even if the child consumes a healthy diet. Similar effects have been observed in mouse models with the metabolic abnormalities persisting across generations. The underlying mechanisms are thought to involve epigenetic inheritance but are poorly defined. We hypothesize that histone methylation in sperm is one such mechanism implicated in transgenerational inheritance of environmentally-induced phenotypes. To test this hypothesis, we are using an existing transgenic mouse model with an altered sperm epigenome. We aim to assess whether there are cumulative effects on histone methylation in sperm resulting from a high-fat diet, and enhanced phenotypes in high-fat fed-transgenic offspring. Transgenic, wildtype littermates and C57BL/6 control males (n=10) were fed either a low- or high-fat diet (10% or 60% kcal fat respectively, from Research Diets) for 12 weeks starting at weaning. Males were phenotypically characterized for glucose and insulin tolerance. They were then bred to 8-weeks old control females on normal chow diet. Offspring fed a normal chow will be phenotyped for metabolic functions similarly to their fathers, and sires sperm epigenome will be assessed by ChIP-sequencing for H3K4me3. Preliminary data show that males on high-fat diet become considerably more obese than the ones on low-fat diet. The obese males show glucose intolerance, insulin insensitivity and elevated glucose levels at fasting. Interestingly, the observed metabolic abnormalities seem exacerbated in the transgenic and their wildtype littermates compared to control.

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POSTER 32

DEVELOPING A BIOINFORMATICS PIPELINE FOR OPTIMIZATION OF SPERM EPIGENOME ANALYSIS IN MICE AND MEN TO BE USED FOR THE IDENTIFICATION OF EPIGENETIC SIGNATURES IN SPERM ASSOCIATED WITH ENVIRONMENTAL PERTURBATION.

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Sperm has a unique chromatin conformation with the majority of somatic histones being replaced with protamines. Thus, unlike a typical ChIP-seq profile generated from targeting a histone modification there are fewer histone peaks and these tend to be distributed over CpG (5'-C-phosphate-G-3') enriched regions. Effects of the paternal environment including stress, diet and toxicants have been linked to negative outcomes for offspring including birth defects and increased risks for complex diseases. These paternal effects may occur via non-genetic inheritance, through epigenetic mechanisms including DNA methylation, post-translational modifications of histones and noncoding RNAs. We hypothesize that, the sperm epigenome in men, specifically histone H3 lysine 4 tri-methylation (H3K4me3), can influence offspring development and health. The challenges in analyzing and quantitating ChIP-seq data from sperm with currently available software is the ability to detect and quantify differences not just in peak enrichment but also the broad domains. Our objective is to develop the most suitable bioinformatics pipeline for semi-quantitative/quantitative comparison of H3K4me3 levels in sperm from fertile and infertile, men of varying folate status, BMI and toxicant exposures. To perform an optimal pre-processing and to address other challenges in data analysis, I am developing an efficient bioinformatics pipeline for analyzing sperm epigenome data, by using currently available tools (Bowtie2, Trimmomatic, Picard tools, MACS2, etc.), to address the challenges of identification of the most reliable peak calling method with appropriate parameters while taking into account the unique chromatin configuration in sperm.

Keywords: Bioinformatics, Chip, Seq, Data Analysis, Pipeline, Epigenomics, Fertility, Infertility, Sperm, Supervised Peak Calling, Machine Learning

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POSTER 33

HISTONE MODIFICATIONS IN GRANULOSA CELLS DURING FOLLICULAR DEVELOPMENT AND OVULATION

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Follicle stimulating hormone (FSH) drives follicular development through altered gene expression in granulosa cells. Fully developed antral follicles respond to the luteinizing hormone (LH) surge, which regulates ovulation through a unique gene expression program. Our preliminary data from chromatin immunoprecipitation (ChIP) assays showed that there was an increase in acetylated histone H3 (H3K27ac) and a decrease in trimethylated histone H3 at lysine 9 (H3K9me3), at 4h post-hCG compared to 48h post-eCG. We hypothesized that the gonadotropins FSH and LH induce histone modifications to regulate the granulosa cell gene expression during follicular development and ovulation, respectively. Granulosa cells were collected from immature superovulated mice at different time-points of follicular development and ovulation. We employed ChIP sequencing (ChIP-Seq) analysis to determine genomewide regulation of histone modifications by FSH in granulosa cells of developing follicles and LH in those of preovulatory follicles. We used antibodies against H3K4me3 (active transcription) and H3K9me3 (repressed transcription) for ChIP assays. After peak calling with MACS2, there were 14137 peaks identified at 0eCG; 14435 peaks at 48eCG and 12141 peaks at 4hCG. The data analysis for H3K4me3 revealed 116 genes that were significant between 0eCG and 48eCG, 9463 genes between 0eCG and 4hCG and 9600 genes between 48eCg and 4hCG (abs[logFC]$\geq$1 and FDR$\leq$0.05). GSEA analysis showed that cellular pathways including cell cycle regulation and differentiation were enriched at the 48eCG and 4hCG time-points. Our interim conclusion is that FSH and LH induce histone modifications in granulosa cells underpinning their proliferation and differentiation. Further bioinformatic analyses are in progress.

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POSTER 34

INTERACTIONS OF PATERNAL FACTORS AND THE USE OF ASSISTED REPRODUCTIVE TECHNOLOGIES: EFFECTS ON OFFSPRING AND EPIGENETIC OUTCOMES

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Proper DNA methylation of sperm is indispensable for fertility and healthy offspring. There is increasing evidence that the sperm epigenome is affected by many factors (diet, smoking etc.) and that abnormal epigenetic patterns can be transmitted to offspring. Not only environmental factors but also genetic factors contribute to the sperm epigenome. An epigenetic regulator, DNMT3L, indirectly participates in *de novo* methylation and is essential for spermatogenesis. In the last few decades, increased infertility has increased use of assisted reproductive technologies (ART). Even though, ART is generally accepted as safe, research indicates an increase in birth and imprinting defects in ART conceived offspring. Our aim is to understand how paternal lifestyle and genetic factors in combination with ART adversely influence offspring development and DNA methylation patterning. We hypothesize that paternal obesity and *Dnmt3L* haploinsufficiency will exacerbate the negative effects of ART on offspring outcomes and DNA methylation.

We use *Dnmt3L* $^{+/-}$ or wildtype C57 male mice fed either with a chow diet (18% kcal) or a high fat diet (60% kcal) for 12 weeks starting from 8-weeks age. At the end of the diet treatments, the sperm are collected for in vitro fertilization and reduced representation bisulfite sequencing. Embryos are collected at mid-gestation (E11.0) to determine embryonic and placental abnormalities as well as DNA methylation changes. This proof of principle mouse model is specifically designed to elucidate the role of sperm epigenetic abnormalities in a clinically relevant multifactorial paternal subfertility/infertility model, associated signatures with the use of ART for reproduction (i.e. more similar to real subfertile/infertile male population).

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POSTER 35

DETERMINING THE LINKS BETWEEN BODY MASS INDEX, FOLATE, HOMOCYSTEINE AND FERTILITY PARAMETERS IN MEN

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INTRODUCTION: Infertility occurs in 15% of couples with male factors implicated in 30-40% of cases. Numerous factors contribute to infertility such as obesity and other lifestyle factors. The role of paternal diet and obesity in reproductive health, specifically folate is unclear. In a mouse model, paternal folate deficiency (FD) was associated with increased birth defects and an altered sperm epigenome (Lambrot et al., 2013).
OBJECTIVE: The objective of this study is to explore the relationship between BMI, folate status, sperm quality, and the heritable sperm epigenome. We hypothesized that levels of folate and overweight status will be associated with altered fertility parameters.
METHODS: In a pilot study, 140 men were recruited at the CReATe fertility centre (IRB approval McGill and U Toronto) for semen and blood collection. Semen samples were analysed for fertility parameters, blood samples for RBC folate and Genetic polymorphisms related to one-Carbon metabolism. Participants were measured for weight and height to calculate BMI.
RESULTS: 12.9% of men were homozygous for the minor allele of the gene MTHFR 677C>T. 62.5% of participants were classified as overweight and obese (n=82/131, BMI $\geq$25). 25% of men had an adequate, not high, RBC folate levels (14/57, 320-1090 nmol/L). Adequate folate levels and obesity were associated with an increased DNA fragmentation index (DFI) (p=0.03, p=0.04, respectively). Overweight men had a reduced motility (p=0.02).
CONCLUSION: This preliminary analysis suggests that folate, overweight status and fertility parameters are interrelated. The suggestions of new links between obesity, folate status and increased DFI are intriguing as high DFI is associated with increased pregnancy loss (Robinson et al., 2012). In our ongoing studies, data on 200 men will confirm these relationships and the link to the sperm epigenome.

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POSTER 36

EFFECTS OF I*N UTERO* AND LACTATIONAL EXPOSURE TO BROMINATED FLAME RETARDANTS ON RAT OVARIAN FUNCTIONS

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Brominated flame retardants (BFRs) are omnipresent in the domestic environment due to their use in textiles, plastics and electronic circuitry. BFRs (predominantly polybrominated diphenyl ethers or PBDEs) bioaccumulate and are detected in ovarian follicular fluid and breast milk. Previous studies from our laboratory have shown harmful effects of PBDEs on the female reproductive system: PBDEs altered granulosa cell function in KGN cells exposed *in vitro*, while *in vivo* exposure to BFRs induced an increase in the number of preantral and antral follicles in dams and accelerated the onset of puberty in their progeny. Here we tested the hypothesis that *in utero* and lactational exposure to BFRs will disrupt ovarian function in the offspring. From preconception to weaning (postnatal day (PND) 21), Sprague-Dawley female rats were fed a diet designed to deliver a BFR mixture reflecting house dust levels at nominal dose 0, 0.06, 20 or 60 mg/kg body weight/day. On PND46 female progeny were killed and ovarian folliculogenesis was examined. There was a trend towards a dose-dependent increase in the number of primary follicles with BFR exposure. The number of multi-oocyte follicles at different stages of folliculogenesis was significantly increased in the 20 and 60 mg/kg/day progeny. A significant increase in the numbers of secondary and antral follicles displaying missing and/or disorganised layers of granulosa cells was also observed in these groups. Thus, early exposure to BFRs has an impact on folliculogenesis. Investigation of the mechanism of action of BFRs on the ovary using gene expression microarrays is ongoing.

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POSTER 37

EFFECTS OF EARLY LIFE EXPOSURE TO CONTAMINATED SEDIMENT IN FISH

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Polycyclic aromatic hydrocarbons (PAHs) are a class of environmental contaminants which are known to be extremely toxic to early life stages (ELS) of fish. The focus of this research is to assess biochemical and organismal-level effects of PAHs on ELS of fish, and to investigate whether DNA methylation plays a role in mediating these effects. Zebrafish embryos and early stage larvae (prior to swim-up) were exposed to sediments collected from control and two PAH-contaminated sites in Lake Saint-Louis, Qc. Individuals exposed to sediment from one of the contaminated sites had significantly higher rates of edema ($p = 0.0366$) and spinal abnormalities ($p=0.0408$). Additionally, significant differences in mean adjusted survival rates were observed between treatments ($p<0.0001$). Levels of global DNA methylation were extremely stable across treatments; average percent DNA methylation ranged from 85.89-87.29% in these samples. A similar result was observed in a zebrafish hepatocyte cell line; PAH congeners benzo[a]pyrene and benzo[k]fluoranthene had no effect on global DNA methylation. In addition to these laboratory experiments we are also assessing effects of exposure to PAH-contaminated sediment in ELS walleye in the field using egg incubation boxes. The use of this *in situ* method, in conjunction with more controlled in vitro and in vivo laboratory-based dosing studies, will be beneficial for assessing the impact that early life exposure to contaminated sediments has on the health of fish.

Funding Sources: Fisheries & Oceans Canada

POSTER 38

EXPOSURE TO A CURRENT-USE FLAME RETARDANT IN JAPANESE QUAIL: EFFECTS ON THE STRESS RESPONSE AND STRESS-RELATED BEHAVIOUR

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Triphenyl phosphate (TPHP) is an organophosphate flame retardant that has been classified as a priority chemical under the Government of Canada's Chemicals Management Plan. Although not considered to be bioaccumulative, this emerging contaminant is still present at high concentrations within biota due to its widespread use as a plasticizer and flame retardant. Preliminary studies have demonstrated that TPHP causes behavioural changes and deformities in zebrafish, however, little is known about this compound's potentially toxic effects in other taxa. We conducted a study in Japanese quail (*Coturnix japonica*) to evaluate possible effects of TPHP in terrestrial wildlife. We exposed chicks to either environmentally relevant levels of TPHP (5 ng/g), safflower oil (control), higher TPHP levels (50 ng/g and 100 ng/g) or a major metabolite of TPHP (DPHP, 100 ng/g) both *in ovo* and orally for the first week of life. Corticosterone levels in blood were measured from 6 day old chicks within three minutes of entering the room to establish baseline values, then again 30 minutes later to evaluate the stress response. These values were compared to behavioural measures of boldness through neophobia and exploratory tasks and to tonic immobility, an extreme fear response. Increasing our understanding of endocrine and behavioural effects of TPHP in birds will support risk assessment to effectively minimize any potential deleterious impacts on wildlife.

Funding sources: Chemical Management Plan, Canada

POSTER 39

ZINC SUPPLEMENTATION REDUCES CYCLOPHOSPHAMIDE INDUCED OXID ATIVE STRESS AND DNA DAMAGE IN MALE GERM CELLS

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Zinc is required for numerous enzymes and DNA binding proteins involved in oxidative stress defense and DNA damage repair. A previous study in our lab suggests that cyclophosphamide (CPA), an alkylating agent and known germ cell toxicant, alters zinc homeostasis in germ cells, contributing to the damaging effects of the drug. We hypothesize that zinc supplementation will protect germ cells from CPA induced oxidative stress and DNA damage. Adult male Sprague Dawley rats received one of four treatments 6 days/week for five weeks: saline, ZnCl2 (20 mg/kg), saline only for the first week then CPA (6 mg/kg) for 4 weeks, or ZnCl2 (20 mg/kg) only for the first week then CPA (6 mg/kg) for 4 weeks. Fluorescent imaging of purified germ cells using CellRox, a marker for reactive oxygen species (ROS), revealed that ROS levels were increased in pachytene spermatocytes from animals treated with CPA compared to controls. Zinc supplementation in CPA treated animals reduced ROS levels to those of controls. A similar trend was observed in round spermatids. DNA damage, assessed by the mean intensity of immunofluorescent staining for ɣH2Ax in testis sections, was increased after CPA treatment and foci sizes were more widely distributed compared to controls. Zinc supplementation resulted in a trend towards a decrease in ɣH2Ax signal when compared to CPA treatment alone and foci size distribution resembled that of controls. Although further studies are needed, these data suggest a role for zinc supplementation in protecting male germ cells against CPA insult.

Funding Sources: Supported by CIHR.

POSTER 40

FETAL EXPOSURE TO GENISTEIN (GEN) AND DI−(2−ETHYLHEXYL) PHTHALATE (DEHP) ALTERS JAK/STAT SIGNALING IN RAT TESTIS

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Introduction and objectives:
Perinatal phases of development have been shown to be particularly sensitive to chemical exposures. Fetal exposure to chemicals with endocrine disruptor properties is believed to predispose males to reproductive abnormalities. The purpose of the current study was to investigate the possibility that a mixture of two common compounds, the phytoestrogen genistein (GEN) and the phthalate plasticizer DEHP, administered to pregnant rats at doses relevant to humans, would change signaling cascades in the testes of their offspring.

Methods:
Pregnant SD rats were gavaged with corn oil (vehicle) or 10 mg/kg/day of DEHP, GEN or their mixture. Male offspring were sacrificed at Postnatal day (PND)3 and 120, and total RNA was extracted from the testes, using offspring from three different dams per treatment. Affymetrix 2.0 ST microarray analysis was conducted by Genome Quebec. Pathway analysis (DAVIDS, Ingenuity, KEGG) were used to identify candidate genes for further validation and expression studies.

Results:
Gene array and pathway analyses revealed that fetal exposure to GEN, DEHP and their mixture resulted in distinct gene expression patterns in the JAK/STAT, PI3K/AKT/mTOR and MAPK pathways, highlighting several genes uniquely disrupted by the mixture. Moreover, different sets of genes were affected in neonatal and adult testes, with GEN+DEHP mixture significantly altered JAK/STAT pathway genes in neonatal testes.

Conclusion:
These data suggest that fetal exposure to an environmentally relevant dose of GEN and DEHP mixture disturbs signaling pathways in neonatal and adult testes, potentially affecting downstream cascades critical for cell cycle and cell survival, which finally may contribute to testicular dysfunction.

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POSTER 41

INVESTIGATION LOSS-OF-LKB1 FUNCTION IN OVIDUCTAL EPITHELIUM AND ITS ROLE IN FACILITATING THE INITIATION OF HIGH-GRADE SEROUS OVARIAN CARCINOMA (HGSOC)

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Ovarian cancer remains one of the most lethal gynecological cancers that affect women today [1]. High-grade serous ovarian carcinoma (HGSOC) is the most common type of ovarian cancer and is most commonly diagnosed at advanced stages when metastasis has occurred [1]. The ovary has traditionally been accepted as the site of initiation but recent research suggests an alternative site of origin; the fallopian tube/oviduct [2]. New evidence proposes that premalignant secretory epithelial cells of the fimbriae (distal oviduct) separate and relocate to the ovary [3]. Investigating the mechanism of how these cells change and translocate is important in determining HGSOC initiation and progression [4]. This will give new insight that will be critical for the development of early effective preventative measures for HGSOC [5].

Lkb1, a known tumor suppressor protein, is a kinase that regulates cell polarity, energy metabolism, apoptosis, cell proliferation and cell cycle arrest [6]. *Lkb1* gene mutations altering its protein function lead to Peutz-Jeghers syndrome which is associated with an increased risk of developing various types of cancers including breast, cervical and ovarian cancer [7]. Approximately 70% of patients with HGSOC show a decrease in Lkb1 protein suggesting that loss-of-Lkb1 plays a role in the mechanism for initiation and progression of HGSOC [8]. Interestingly, a previous study from our lab showed that live epithelial cells spontaneously detach from their epithelial layers in *Lkb1* null embryos [9]. We hypothesize that loss-of-Lkb1 facilitates cell shedding in precancerous epithelial cells of the oviduct which then translocate to the ovary.

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POSTER 42

ESTABLISHMENT OF OVIDUCT ORGANOID CULTURES TO STUDY THE ROLE OF *LKB1* IN EPITHELIAL MORPHOGENESIS AND HOMEOSTASIS

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LKB1 (Liver Kinase B1) is a tumour suppressor involved in energy sensing, cell cycle and cell polarity regulation. The deletion of *Lkb1* in mice is embryonic lethal and causes cell extrusion from non-transformed epithelia in the blastocyst stage [1]. On the other hand, *LKB1* loss is associated with early tumorigenesis of High Grade Serous Carcinoma (HGSOC) [2], which has the highest mortality rate amongst gynaecological disorders. Interestingly, the current model of HGSOC is that malignant cells originate from the oviductal epithelial cells [3]. This model suggests that the oviductal epithelial cells somehow translocate to the ovary during HGSOC tumorigenesis. We hypothesize that the **loss of *LKB1* in the oviductal epithelium could facilitate cell translocation by modulation of epithelial morphogenesis and cellular metabolism**.

In this project, we aim to understand the role of LKB1 in epithelial morphogenesis using *in vitro* 3D culture systems of two cell types: embryonic stem cells (ESCs) and oviduct luminal epithelial cells. Our preliminary results showed that *Lkb1 -/-* ESCs had changes in cell size, cell cycle phase lengths, cell doubling time, and metabolism. When cultured in 3D conditions, they formed larger rosettes and underwent a greater amount of cell death. This suggests that LKB1 loss has detrimental effects over the course of differentiation, and possibly interferes with epithelial morphogenesis. For oviduct epithelial cell/organoid cultures, we are currently optimizing tissue dissociation, culture, and passage protocols in both 2D and 3D conditions.

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References:

[1] D. Krawchuk, S. Anani, N. Honma-Yamanaka, S. Polito, M. Shafik, and Y. Yamanaka, “Loss of LKB1 leads to impaired epithelial integrity and cell extrusion in the early mouse embryo.,” *J. Cell Sci.*, vol. 128, no. 5, pp. 1011–22, 2015.

[2] S. H. L. George, a Milea, R. Sowamber, R. Chehade, a Tone, and P. a Shaw, “Loss of LKB1 and p53 synergizes to alter fallopian tube epithelial phenotype and high-grade serous tumorigenesis.,” *Oncogene*, vol. 35, no. January, pp. 1–10, 2015.

[3] R. Perets *et al.*, “Article Transformation of the Fallopian Tube Secretory Epithelium Leads to High-Grade Serous Ovarian Cancer in Brca ; Tp53 ; Pten Models,” *Cancer Cell*, vol. 24, no. 6, pp. 751–765, 2013.

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