Centre for the Study of/Centre d'études sur la Reproduction

HUMAN REPRODUCTION AND DEVELOPMENT

BREAKTHROUGHS IN REPRODUCTION AND DEVELOPMENT

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NOUVELLES AVANCÉES EN REPRODUCTION ET DÉVELOPPEMENT

Research Day

Centre for the Study of Reproduction (CSR) at McGill &
the Human Reproduction and Development Axis
of the Research Institute of the MUHC

Wednesday, May 7, 2014

New Residence Hall
3625, avenue du Parc
Montréal, Québec

Centre universitaire de santé McGill
McGill University Health Centre

McGill

Centre for the Study of/Centre d'études sur la Reproduction

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8:00-9:00 Registration and coffee / Poster set-up

9:00-9:15 Opening remarks: **Dr. Hugh Clarke**

9:15-9:45 **Dr. Florence Couteau, Department of Biology, McGill University**, *"Epigenetic memory of DNA damage repair in germ stem cell affects fertility"* – introduced by Dr. Teruko Taketo

9:45-10:30 Oral presentations (Chairs: Gurpreet Manku and Karl Vieux)
O-01. Ellis Fok, *"The Ubiquitin Ligase Huwe1 Is Required For Establishment And Maintenance Of Sperm Progenitor Cells"*
O-02. Keith Siklenka, *"Histone H3 Lysine 4 Di-Methylation In Sperm Is Implicated In Embryo Development And Transgenerational Epigenetic Inheritance"*
O-03. Serge McGraw, *"Transient Dnmt1 Suppression Reveals Hidden Heritable Marks In The Genome"*

10:30-11:00 Health Break / Posters

11:00-11:30 Oral presentations (Chairs: Pavine Lefèvre and Keith Siklenka)
O-04. Malena Rone, *"Mitochondrial Metabolic Activity Assists With Regulation Of Steroid Production In MA-10 Mouse Leydig Cells"*
O-05. Geneviève Plante, *"High Density Lipoproteins And Binder Of Sperm Proteins: A New Piece Of The Puzzle In Murine Sperm Capacitation?"*

11:30-12:15 **Dr. Amander Clark, Department of Molecular, Cell and Developmental Biology, Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research, UCLA**, *"Arginine methylation is required for germ line development and ground state pluripotency"* – introduced by Dr. Makoto Nagano

12:15-13:45 Lunch / Posters
P-01. Laleh Abbassi, "*Role Of The Hippo Signalling Pathway In Oocyte Growth"*
P-02. Cécile Adam, "*SP1 and AP2 Transcription Factors Regulate Cx26*

Gene Expression in the Epididymis."

P-03. Yasaman Aghazadeh, *"Induction Of Androgen Formation In The Male By A Peptide Blocking 14-3-3ε Protein Adaptor And Mitochondrial Vdac1 Interactions"*

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P-16. Steven Jones, "*Long Term Effects Of Low Dose Prenatal Exposure To Endocrine Disruptors On Male Reproduction And Development*"

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P-21. Marion Mandon. *"Unravelling The Mystery Of Basal Cells: Isolation And Characterization Of Rat Epididymal Basal Cells"*
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15:30-16:15 **Dr. Humphrey Yao, Laboratory of Developmental and Reproductive Toxicology, National Institute of Environmental Health Sciences**,*"Making an Ovary: From Cell Fate Determination to Folliculogenesis"* – introduced by Dr. Cristian O'Flaherty

16:15-16:30 Awards Presentation and Concluding Remarks: **Dr. Hugh Clarke**

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NOUVELLES AVANCÉES EN REPRODUCTION ET DÉVELOPPEMENT

Journée de recherche 2014

Centre d'études sur la reproduction (CER) à McGill &
l'axe de la reproduction et du développement humain de l'IR-CUSM

le mercredi 7 mai 2014
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16 h 15 Présentation de prix et mot de conclusion : **Dr. Hugh Clarke**

16 h 30 Démontage des affiches

DR. AMANDER CLARK

DEPARTMENT OF MOLECULAR CELL AND DEVELOPMENTAL BIOLOGY, ELI AND EDYTHE BROAD CENTER OF REGENERATIVE MEDICINE AND STEM CELL RESEARCH, UCLA.

ARGININE METHYLATION IS REQUIRED FOR GERM LINE DEVELOPMENT AND GROUND STATE PLURIPOTENCY.

Protein arginine methyltransferase 5 (PRMT5) is a type II arginine methyltransferase that catalyzes the symmetrical di-methylation of arginine (SDMA) amino acids in proteins. PRMT5 has diverse roles in cell biology and has been implicated in regulating stem cell biology, reprogramming, cancer and neurogenesis. In stem cells PRMT5 prevents naïve stem cell differentiation, however its role in ground state pluripotency and mammalian germline formation is unknown. We developed an inducible *Prmt5* knock out (iPKO) embryonic stem cell (ESC) model and show that Prmt5 safeguards ground state pluripotency by enabling appropriate constitutive and alternate splicing. We show that deleting Prmt5 is not compatible with survival, causing an increase in unrepaired double strand breaks coupled with abnormal splicing of mRNA processing genes, DNA damage repair genes, and chromatin modifies that ultimately lead to changes in the global state of chromatin and apoptosis. Using a conditional deletion of Prmt5 in primordial germ cells (PGCs) we show that *Prmt5* deficient PGCs undergo apoptosis between embryonic (E) day E10.5-E13.5 as a consequence of PGCs exiting the cell cycle. Using the ESC to PGC like cell (PGCLC) differentiation model we show that deleting Prmt5 in epiblast like cells leads to the formation of fewer PGCLCs. Taken together, our study demonstrates that arginine methylation downstream of PRMT5 is a key regulator of cell survival in ground state pluripotency and is required for the earliest stages of germ line development in mammals.

DR. HUMPHREY YAO

LABORATORY OF DEVELOPMENTAL AND REPRODUCTIVE TOXICOLOGY, NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES

MAKING AN OVARY: FROM CELL FATE DETERMINATION TO FOLLICULOGENESIS

The molecular pathways that drive the differentiation of somatic cell populations in the testis and ovary have been the subjects of intensive research over the past decade. It is now clear that ovarian differentiation is a coordinate event driven by secreted factors including R-spondin1, WNT4, and follistatin and transcriptional regulators such as β-catenin and FOXL2. These factors direct bipotential somatic cell lineages toward an ovarian fate and simultaneously suppress the emergence of testis-determining processes. This presentation will summarize the molecular pathways responsible for establishment of the ovary and discusses the current hypotheses on the origin(s) of somatic cell lineages and how these somatic cells acquire the characteristics necessary for their functions during ovarian development and maintenance.

ORAL PRESENTATIONS

THE UBIQUITIN LIGASE HUWE1 IS REQUIRED FOR ESTABLISHMENT AND MAINTENANCE OF SPERM PROGENITOR CELLS

Kin Lam FOK[1], Rohini BOSE[1], Wenming XU[2], Martine CULTY[1], Hsiao CHANG CHAN[3], Antonio IAVARONE[4], Anna LASORELLA[4], Simon S. WING[1]

[1]Department of Medicine, McGill University, Montreal, Quebec, Canada [2]Laboratory of Reproductive Medicine, Sichuan University, Chengdu, China [3]School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong [4]Institute for Cancer Genetics, Columbia University Medical Center, New York, NY, USA

Spermatogenesis is supported by spermatogonial stem cells (SSCs) and differentiating spermatogonia. These sperm progenitor cells are derived from gonocytes in the first wave of spermatogenesis. However, the mechanism underlying their establishment and maintenance remains largely unknown. In our previous studies, we have identified an E3 ubiquitin ligase, Huwe1, and demonstrated its translocation from cytoplasm to nucleus during the transition from gonocyte to sperm progenitor cells. More recently, we have inactivated Huwe1 specifically in the primordial germ cells (Huwe1$^{flox/Y}$ Ddx4-Cre^{+}) and differentiating spermatogonia (Huwe1$^{flox/Y}$ Stra8-Cre^{+}) to explore the role of Huwe1 in distinct sperm progenitor cells. Our results showed that Huwe1$^{flox/Y}$ Ddx4-Cre^{+} mice were infertile while Huwe1$^{flox/Y}$-Stra8-Cre^{+} mice were subfertile. Morphological analysis showed complete depletion of germ cells in adult Huwe1$^{flox/Y}$ Ddx4-Cre^{+} testes and a remarkable decrease in primary spermatocytes in adult Huwe1$^{flox/Y}$ Stra8-Cre^{+} testes. Immunostaining of germ cell markers, Ddx4 and Tra98, in different developmental stages of Huwe1$^{flox/Y}$ Ddx4-Cre^{+} testes revealed a progressive degeneration of germ cells from 6 dpp onwards. Gene expression profiling by microarray and realtime PCR showed decreases in both self-renewal markers and differentiation markers in 6 dpp Huwe1$^{flox/Y}$ Ddx4-Cre^{+} testes. BrdU incorporation assay and immunostaining of gonocyte marker, Foxo1, showed a significant decrease in the mitotic re-entry of gonocyte and perturbation in transiting to sperm progenitor cells in 3 dpp Huwe1$^{flox/Y}$ Ddx4-Cre^{+} testes. Knockout or silencing of Huwe1 in the testis or spermatogonial stem cell line, C18-4, decreases the protein level of Foxo1, which is essential for early germ cell development. Taken together, loss of Huwe1 perturbs the establishment and maintenance of sperm progenitor cells in the first wave of spermatogenesis. Further studies are being undertaken to decipher the mechanism(s) underlying the control of early germ cell development by Huwe1.

HISTONE H3 LYSINE 4 DI-METHYLATION IN SPERM IS IMPLICATED IN EMBRYO DEVELOPMENT AND TRANSGENERATIONAL EPIGENETIC INHERITANCE

Keith SIKLENKA, Serap ERKEK[1], Maren GODMANN[2], Romain LAMBROT[2], Christine LAFLEUR[2], George CHOUNTALOS[2], Tamara COHEN[2], Matthew SUDERMAN[2], Mike HALLETT[2], Serge MCGRAW[2], Donovan CHAN[2], Jacquetta TRASLER[2], Antoine PETERS[1] and Sarah KIMMINS[2]

[1]Friedrich Miescher Institute for Biomedical Research, Switzerland; [2]McGill University, Montreal, Quebec, Canada

It has long been known that the health of offspring can be influenced through paternal effects however, the underlying mechanisms remain unclear. Sperm histones, previously thought to be retained at random and without function, are associated with CpG islands and hypomethylated DNA (Erkek et al 2013). Moreover, activating histone modifications such as histone 3 lysine 4 (H3−K4) methylation were found localized to promoters of genes implicated in embryonic development (Brykczynska et al 2010; Hammoud et al 2009). We hypothesize that the epigenetic marks on retained sperm histones serve to influence the health and development of offspring. Therefore, we altered the mouse sperm epigenome through overexpression of the histone demethylase KDM1A specifically in the testes. Characterization of offspring sired by heterozygous $KDM1A^{tg/+}$ males revealed reduced survivability and a range of developmental defects. Strikingly, the severe developmental phenotype was observed in offspring sired by non-transgenic descendants and persisted for three generations. These data suggests that inherited germ−cell epimutations may resist reprogramming for multiple generations before being reset. Analysis of the sperm epigenome of transgenic fathers by chromatin immunoprecipitation combined with genome sequencing (ChIP−Seq) revealed specific reductions of H3K4me2 at transcriptional start sites of over 2000 genes. Gene ontology analysis of these regions showed significant enrichment for genes associated with metabolic process, development and patterning. Moreover, Sequenome MassArray was used to analyze DNA methylation of transgenic and non−transgenic sperm at candidate genes; however, no significant differences were observed. Here we show that sperm histone modifications, particularly H3K4me2, are important for guiding offspring development across multiple generations.

TRANSIENT DNMT1 SUPPRESSION REVEALS HIDDEN HERITABLE MARKS IN THE GENOME

Serge MCGRAW[1], Jacques X. ZHANG[1], Mena FARAG[1], Donovan CHAN[1], Maxime CARON[3], Carolin KONERMANN[2], Christopher C. OAKES[2], K. Naga MOHAN[4], Christoph PLASS[2], Tomi PASTINEN[3], Guillaume BOURQUE[3], J. Richard CHAILLET[4], Jacquetta M. TRASLER[1]

[1]Research Institute at the Montreal Children's Hospital of the MUHC, Departments of Pediatrics, Human Genetics and Pharmacology & Therapeutics, McGill University, Montreal, Quebec, Canada, [2] German Cancer Research Center, Heidelberg, Germany, [3]McGill University and Genome Quebec Innovation Centre, Montreal, Quebec, Canada; [4]University of Pittsburgh, Pittsburgh, PA, USA.

Genome-wide demethylation and remethylation of DNA during early embryogenesis is essential for mammalian development and genome integrity. Imprinted germline differentially methylated domains (gDMDs) established by sex-specific methylation in either male and female germ cells, must escape these dynamic changes and sustain precise inheritance of both methylated and unmethylated parental alleles. To identify gDMD-like sequences with the same epigenetic inheritance properties, we used a modified embryonic stem (ES) cell line that replicates the embryonic demethylation and remethylation waves, through the inducible repression of *Dnmt1*. Six days of doxycyline (DOX) treatment of *Dnmt1*$^{tet/tet}$ ES cells repressed DNMT1, the main methylation maintenance enzyme, and produced a significant reduction in genomic methylation. Most loci regained their original methylation three weeks after DOX removal, with the notable exception of the gDMDs of imprinted genes. By performing comprehensive DNA methylation profiling, using recombinant MBD2 methyl-CpG immunoprecipitation (MCIp) with mouse CpG island microarrays, as well as reduced representation bisulfite sequencing (RRBS), we showed that the transient *Dnmt1* suppression exposed gDMD-like sequences requiring continuous DNMT1 activity to sustain a highly methylated state. Remethylation of these sequences was also impeded in a mouse model of transient DNMT1 loss. These novel regions, possessing heritable epigenetic features similar to imprinted-gDMDs may be associated with normal development and when disrupted associated with disease states. The present study presents new perspectives on DNA methylation heritability during embryo development that extends beyond conventional imprinted-gDMDs.

MITOCHONDRIAL METABOLIC ACTIVITY ASSISTS WITH REGULATION OF STEROID PRODUCTION IN MA-10 MOUSE LEYDIG CELLS

Malena B. RONE, Andrew S. MIDZAK, Daniel B. MARTINEZ-ARGUELLES, Vassilios PAPADOPOULOS

Research Institute of the McGill University Health Centre and the Department of Medicine, McGill University, Montreal, Quebec, Canada

Mitochondria are home to many cellular processes, including oxidative phosphorylation, fatty acid metabolism, and in steroid synthesizing cells, cholesterol import and metabolism to pregnenolone. The formation of macromolecular protein complexes aids in the regulation and efficiency of these mitochondrial functions, though due to their dynamic nature are hard to identify. To overcome this problem we utilized Blue-Native polyacrylamide gel electrophoresis (BN-PAGE) coupled to mass spectrometry on isolated mitochondria from control and hormonally stimulated mouse MA-10 Leydig cells. The data obtained identified the presence of a number of qualitatively similar mitochondrial protein machineries, under control and hCG-stimulated conditions. In addition, quantitative differences were observed in mitochondrial complex formation after hormone stimulation as compared to control cells. A prominent decrease of mitochondrial proteins involved in fatty acid import into the mitochondria was observed. From these results we implied that mitochondrial β-oxidation is not essential for steroidogenesis. To confirm this we inhibited fatty acid import utilizing the carnitine palmitoyltransferase Ia (CPT1a) inhibitor etoxomir, resulting in an increase in steroid production after 24 hour incubation of the cells with the drug. Moreover, etoxomir induced a decrease in oxygen consumption with an increase in extracellular acidification, confirming the inhibition of β-oxidation. A shift towards glycolysis with no observed lost ATP production was also observed. These results suggest that changes in the metabolic profile of the mitochondria in steroidogenic cells can function as a potential regulator in cholesterol import and steroid production. We propose that upon hormonal stimulation, the mitochondria efficiently import cholesterol at the expense of other lipids necessary for energy production resulting in their specialization for steroid biosynthesis.

HIGH DENSITY LIPOPROTEINS AND BINDER OF SPERM PROTEINS: A NEW PIECE OF THE PUZZLE IN MURINE SPERM CAPACITATION?

Geneviève PLANTE and Puttaswamy MANJUNATH

Centre de recherche HMR, Université de Montréal, Montréal, Québec, Canada

Binder of SPerm (BSP) proteins are ubiquitous amongst mammals and are exclusively expressed in male genital tract. The main function associated with BSP proteins is their ability to promote sperm capacitation. Two genes (*Bsph1* and *Bsph2*) coding for BSP proteins have been identified in murine epididymis. Using recombinant proteins, our team demonstrated that murine rec-BSPH1 and rec-BSPH2 could bind to epididymal sperm membranes, but only rec-BSPH1 had the ability to promote sperm capacitation *in vitro*. The goal of the present study was to evaluate the possible role of native BSP proteins on murine sperm capacitation *in vitro*. To do so, we tested the effect of Fabs, $F(ab')_2$ and antibodies raised against murine BSP proteins on capacitation induced by High Density Lipoproteins (HDL) and Bovine Serum Albumin (BSA). Results obtained show that antibodies and $F(ab')_2$ can block capacitation induced by both HDL and BSA, but Fabs can only block capacitation induced by HDL. These results suggest that a specific interaction between HDL and BSP proteins in mouse could be necessary for sperm capacitation. In bovine, BSP proteins, upon interaction with HDL, are removed from sperm membrane and induce a cholesterol and phospholipid efflux. In mice however, no decrease in the amount of BSP proteins on sperm surface was observed following incubation with HDL suggesting a different mechanism. These results seem to indicate that BSP proteins in mice could be important for sperm capacitation. (Supported by FRQS, NSERC and CIHR)

IMPACT OF L-CARNITINE SUPPLEMENTATION DURING VITRIFICATION ON MITOCHONDRIAL DISTRIBUTION AND ATP LEVELS IN THE MOUSE OOCYTES VITRIFIED AT THE GERMINAL VESICLE STAGE, THAWED AND THEN MATURED IN VITRO

Adel R. MOAWAD[1,2,4,5], Baozeng XU[2,4], Seang Lin TAN[1,4] and Teruko TAKETO[1,2,3,4]

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Oocyte cryopreservation has a wide range of potential applications in the field of assisted reproductive technologies (ART). Most of the studies so far have been focused on the cryopreservation of mature oocytes at the metaphase II (MII). MII-oocytes, however, are prone to the damage inflicted by the freezing procedure, such as abnormal MII-spindle assembly. Cryopreservation of germinal vesicle (GV) oocytes could be an alternative method for ART. The rate of blastocyst development from cryopreserved GV-oocytes, however, remains low due to the requirement of in vitro maturation (IVM), which results in less competent oocytes for embryonic development than in vivo ovulated oocytes. We have previously reported that supplementation of L-carnitine (LC) during vitrification and IVM of mouse GV-oocytes increases the success rate of pre-implantation development following IVF. Furthermore, we have found that while half of the vitrified GV-oocytes show abnormal spindle assembly after IVM, LC supplementation during both vitrification and IVM significantly improves MII-spindle assembly to an extent comparable to unvitrified control oocytes. The goal of current study is to elucidate the mechanisms by which LC exerts its beneficial effect on the MII-spindle assembly by examining mitochondrial distribution and ATP levels in the IVM-oocytes of both B6 and (B6.DBA)F1 strains. The results were consistent between the two mouse strains. Our results show that fine aggregates of mitochondria, detected by staining with MitoTracker-green fluorescence, are concentrated in the peripherally (polarized) and evenly distributed in the remaining cytoplasm of unvitrified control MII-oocytes. After vitrification at the GV-stage followed by IVM, a significantly lower percentage of oocytes showed polarized mitochondrial distribution and the average fluorescence intensity was lower compared to the unvitrified control oocytes. Supplementation of both vitrification and IVM media with LC at 0.6 mg/ml significantly increased the percentage of oocytes with normal mitochondrial distribution as well as the average fluorescence intensity compared to the vitrified control, and reached the levels comparable to the unvitrified control. By contrast, vitrification of GV-oocytes did not change the ATP levels in MII-oocytes irrespective of LC treatment. These results suggest that LC supplementation during vitrification and thawing of GV-oocytes minimizes the cytoplasmic damage which influences mitochondrial distribution in the MII-ocytes. The local concentration of mitochondria, rather than the average of ATP levels, may be critical for proper spindle assembly during the MI-MII transition. Supported by Mitacs Elevate Post Doctoral Fellowship to ART and OriginElle Fertility Clinic and Women's Health Centre.

EXPRESSION OF DNA DAMAGE RESPONSE GENES IS UP REGULATED IN LATE-CLEAVED PORCINE EMBRYOS NEAR THE MATERNAL-TO-ZYGOTE TRANSITION PERIOD BUT NOT IN DAY 7 BLASTOCYSTS

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There are many studies showing that fast-cleaving embryos have superior developmental capacity to reach the blastocyst stage compared to slow-cleaving embryos. In our previous studies we have demonstrated that nuclei of fast-cleaving porcine embryos produced by either IVF or SCNT have lower number of H2AX139ph foci, which are markers of DNA double-strand breaks, compared to those of slow-cleaving embryos. These data suggest that genome integrity is an important component affecting early embryo cleavage kinetics and developmental capacity to blastocyst stage. In the present study we assessed transcript abundance of a number of genes involved in two different DNA-damage repair pathways; the homologous recombination (HR) and the nonhomologous end-joining (NHEJ), as well as cell cycle checkpoint genes (CHECK1 and CHECK2) at different stages of in vitro development of fast- and slow-cleaving porcine embryos produced by parthenogenetic activation (PA). Cleavage was accessed at 24 h and 48 h after PA to separate fast- and slow-cleaving embryos, respectively, which were then transferred to a new culture dish. Embryos from each group were collected on day 3 (30 embryos per group), day 5 (20 embryos per group) and day 7 (10 embryos per group) after PA for RNA extraction followed by qRT-PCR to assess transcripts abundance. The experiment was repeated three times and all samples were analysed in duplicate. Total RNA was extracted using the PicoPure RNA isolation Kit (Life Technologies) and cDNA was synthesised using the SuperScript VILO cDNA Synthesis Kit (Life Technologies). Relative mRNA abundance was normalised to the levels of two reference genes (Beta actin and histone H2A). Significantly higher levels of transcripts for RAD51, RAD52 and RPA1 (HR pathway) and LIG4 and XRCC6 (NHEJ pathway) were detected in slow-cleaving compared to fast-cleaving embryos on day 3 of development. Similarly, slow-cleaving embryos had higher mRNA abundance of additional genes of both HR (ATM, ATR, RAD51, RAD52, BRCA1 and MRE11A) and NHEJ (PRKDC, XRCC6, XRCC5, LIG4, XRCC4 and TP53BP1) pathways on day 5 of development

CLASS-SPECIFIC HISTONE DEACETYLASE (HDAC) INHIBITORS HAVE DIFFERENTIAL ADVERSE EFFECTS ON CHONDROGENESIS AND EARLY OSTEOGENESIS IN MURINE LIMB BUDS IN VITRO

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Histone deacetylases (HDACs) regulate the acetylation of both histone and non-histone proteins and are separated into four classes, I to IV.Several studies have shown a correlation between HDAC inhibition and skeletal defects. However, the role of each class in mediating these effects remains unknown. Limb formation requires the expression of specific markers; chondrocytes express Sox9 and Collagen2a1 whereas Runx2 and Collagen10a1 are two key markers of early osteogenesis. Our goal here was to test the hypothesis that class-specific HDAC inhibitors will have differential effects on limb chondrogenesis and osteogenesis.
We cultured embryonic forelimbs from Col2a1-ECFP and Col10a1-mCherry transgenic reporter mice in the presence of class-specific HDAC inhibitors for 6 days: MS275, targeting Class I/Hdac1, MC1568, targeting Class II/Hdac4/6 and Sirtinol, targeting Class III/Sirt2.

MS275-exposed limbs exhibited a decrease in *Col2a1* expression compared to control after 1 day; this difference was maintained until day 6. *Col10a1* expression was completely absent in MS275-treated limbs. In contrast, at an equimolar concentration, MC1568 had minimal effects on *Col2a1* expression. Sirtinol-exposed limbs were not different from control at 1 day. After 3 days, *Col2a1* expression was decreased and after 6 days, a drastic regression was observed in *Col2a1*. *Col10a1* was not expressed in Sirtinol-treated limbs at any timepoint.

Thus, results suggest that Class I HDACs play a major role in limb development whereas inhibition of class II HDACs alone had little impact. Interestingly, class III HDACs inhibition caused the degeneration of differentiated chondrocytes suggesting they have a distinct mechanism of developmental toxicity. These studies were funded by CIHR and FRQS.

POSTER PRESENTATIONS

POSTER 1

ROLE OF THE HIPPO SIGNALLING PATHWAY IN OOCYTE GROWTH

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In most cases, cell growth and the cell division cycle are closely coupled. In the growing oocyte, however, these processes are uncoupled, thus permitting a single very large cell to be produced. How cell-cycle progression is prevented in growing oocytes is not known. Hippo, a highly conserved signalling pathway, has recently been identified in cells. When Hippo is 'OFF', the transcription factor YAP becomes dephosphorylated, moves into the nucleus, and drives the cell cycle. When Hippo is 'ON', YAP becomes phosphorylated and excluded from the nucleus, and the cell cycle becomes arrested. Therefore, we hypothesize that the Hippo pathway promotes oocyte growth by phosphorylating YAP and thereby preventing resumption of cell cycle progression. RT-PCR and immunoblotting results showed that growing and fully grown oocytes express *Yap* RNA and YAP protein. We also found that YAP was phosphorylated at a specific site (S127) and that oocytes express *Lats1* which encodes the kinase that phosphorylates Yap at this site. Using Immunofluorescence, we found that YAP was excluded from the oocyte nucleus, as predicted based on its phosphorylation; control experiments confirmed that the antibody detected nuclear YAP in other cells. To identify the mechanism that regulates LATS1 activity and hence YAP phosphorylation, we manipulated the levels of cyclic AMP, which activates protein kinase A (PKA). Reducing external cAMP caused dephosphorylation of YAP in fully grown oocytes. This suggests that PKA regulates LATS1 in fully grown oocytes. However, increasing external cAMP did not elevate Yap phosphorylation in growing oocytes. Our results demonstrate that dephosphorylation of YAP on S127 is not sufficient to trigger its nuclear translocation, suggesting that other factors also regulate its location in the oocyte. Taken together, these results indicate that the Hippo pathway is ON in growing and fully grown oocytes, consistent with the view that inhibiting YAP is necessary to prevent cell-cycle progression.

POSTER 2

SP-1 AND AP-2 TRANSCRIPTION FACTORS REGULATE CX26 GENE EXPRESSION IN THE EPIDIDYMIS

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Connexins (Cxs) are responsible for forming gap junctions between cells. Our laboratory previously reported that epididymal *Cx26* is expressed only in young animal. The regulation of the *Cx26* gene is in the epididymis is unknown. The present objective was to elucidate the mechanisms regulating the *Cx26* gene in the rat epididymis through the characterization of its promoter. RLM-RACE revealed a single transcription start site (tss) at position -3829 relative to the ATG. Computational analysis revealed several AP-2 and SP1 binding sites located 5' to the tss. A 1.7kb fragment of the *Cx26* promoter was amplified and cloned into pGL3 vector containing a luciferase reporter gene. Transfection of the construct in epididymal RCE cells indicated that the promoter contained sufficient information for transactivation of the reporter gene. Several deletion constructs were generated and transfected into RCE cells. Luciferase assays revealed the presence of two DNA response elements necessary for the expression of *Cx26*: an AP-2/SP-1 site and an SP-1 site. The implication of the two sites was confirmed by directed mutagenesis. ChIP analysis confirmed that these factors bind to the promoter. ChIP analysis and qPCR of DNA from young and pubertal animals indicated that binding of the AP-2 and SP-1 decrease with age when *Cx26* mRNA levels decrease. Results indicate that the *Cx26* gene is regulated by AP-2 and SP-1. The elucidation of these mechanisms will provide a better understanding of processes implicated in the differentiation of the epididymal epithelium. Supported by NSERC.

POSTER 3

INDUCTION OF ANDROGEN FORMATION IN THE MALE BY A PEPTIDE BLOCKING 14-3-3E PROTEIN ADAPTOR AND MITOCHONDRIAL VDAC1 INTERACTIONS

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Low testosterone (T) is a major cause of male hypogonadism and infertility and is linked to mood changes, osteoporosis, reduced bone-mass index, and aging. The treatment of choice, T-replacement therapy, is linked with increased risk for prostate cancer and luteinizing hormone suppression, and shown to lead to reduced fertility, cardiovascular diseases and higher mortality rates. In search of the mechanisms regulating T synthesis in the testes, we identified the 14-3-3ε protein as a negative regulator of steroidogenesis. 14-3-3ε interacts with the outer mitochondrial membrane voltage-dependent anion channel (VDAC1) protein and translocator protein (TSPO), forming a scaffold that limits the availability of cholesterol for steroidogenesis. Peptides were developed using a sequence of HIV transcription factor I conjugated to 14-3-3 motif containing VDAC1S167. These peptides compete with endogenous VDAC1 and blocked 14-3-3ε-VDAC1 interactions *in vitro* and *in vivo*, leading to increased serum and intratesticular T levels in adult male rats. These peptides also rescued intratesticular and serum T formation in adult male rats treated with GnRH antagonist, which dampened LH and T production. These results unveiled a novel mechanism regulating androgen biosynthesis and suggest that the identified peptide is a potential therapy for primary hypogonadism and maintaining physiological T levels without exogenous administration of T.

POSTER 4

INITIATION OF HIPPO SIGNALING IS LINKED TO POLARITY RATHER THAN CELL POSITION IN THE PREIMPLANTATION MOUSE EMBRYO

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In the mouse embryo, asymmetric divisions during the 8-16 cell division generate two cell types, polar and apolar cells, which are allocated to outer and inner positions, respectively. This outer/inner configuration is the first sign of formation of the first two cell lineages, trophectoderm (TE) and inner cell mass (ICM). Outer polar cells become TE and give rise to the placenta, while inner apolar cells become ICM and give rise to the embryo proper and yolk sac. Here, we analyzed the frequency of asymmetric divisions during the 8-16 cell division and assessed the relationships between cell polarity, cell and nuclear position, and Hippo signalling activation, the pathway that initiates lineage-specific gene expression in 16-cell embryos. While the frequency of asymmetric divisions varied in each embryo, we found that more than six blastomeres divided asymmetrically in most embryos. Interestingly, many apolar cells in 16-cell embryos were located at outer positions while only one or two apolar cells were located at inner positions. Live imaging analysis showed that outer apolar cells were eventually internalized by surrounding polar cells. Using isolated 8-cell blastomeres, we carefully analyzed the internalization process of apolar cells and found indications of higher cortical tension in apolar cells than in polar cells. Lastly, we found that apolar cells activate Hippo signalling prior to taking inner positions. Our results suggest that polar and apolar cells have intrinsic differences that establish outer/inner configuration and differentially regulate Hippo signalling to activate lineage-specific gene expression programs.

POSTER 5

THE CLAUDIN FAMILY OF TIGHT JUNCTION PROTEINS IS REQUIRED FOR CONVERGENT EXTENSION AND APICAL CONSTRICTION DURING NEURAL TUBE CLOSURE

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Neurulation is a critical developmental process that converts the flat neural plate into a closed neural tube. Throughout this process the neural plate epithelium undergoes extensive remodeling including cell intercalation and apical constriction. These events require the maintenance of cell interactions within the neural epithelium through intercellular junctions. The most apical of these junctions are tight junctions (TJs), where members of the claudin family regulate apical-basal cell polarity and cell adhesion, and link the TJ to the actin cytoskeleton. We hypothesize that claudins are required for coordinating the morphogenetic events that drive neural tube closure. To test our hypothesis, we used the C-terminal domain of Clostridium perfringens enterotoxin (cCPE) to remove a subset of claudins from the neural plate of chick embryos. 100% of treated embryos had open neural tube defects (NTDs) that could not be rescued by folic acid supplementation. We confirmed that cCPE specifically removed Claudin-3, -4, and -8 but not Claudin-1 from TJs in chick embryos. *In situ* hybridization analysis of gene expression in the ectoderm and along anterior-posterior boundaries of the neural tube suggested that NTDs were not due to defects in ectoderm differentiation or patterning. cCPE-treated embryos displayed defects consistent with a disruption of convergent extension: a shortened anterior-posterior axis, a reduced length-to-width ratio, misshapen somites, and a broadened notochord. Transmission electron microscopy analysis of cCPE-treated embryos showed that midline cells failed to undergo apical constriction. These results suggest that claudins are required for two morphogenetic events that drive neural tube closure – convergent extension and apical constriction.

POSTER 6

TESTOSTERONE REGULATES THE EXPRESSION AND DISTRIBUTION OF CATHEPSIN D, PROSAPOSIN AND SORTILIN IN THE RAT CAUDA EPIDIDYMIDIS

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The epididymis is implicated in sperm maturation by remodeling the sperm plasma membrane. Lysosomal proteins secreted by the epididymal epithelium may play a role in this process. Here we analyzed the expression of cathepsin D (catD), prosaposin (PSAP) and sortilin in the cauda epididymis of intact rats and rats subjected to castration followed or not by testosterone replacement. CatD is a lysosomal hydrolase which is secreted by principal cells into the epididymal lumen, and whose secretion may be associated with PSAP. PSAP is nonenzymic protein that is trafficked in a monomeric form (70kDa) to the lysosomes or secreted as oligomers (250kDA). Sortilin is a TGN-resident protein implicated in the lysosomal sorting of PSAP and catD. Immunoblotting with anti-catD-ab showed an increase in the expression and secretion of this protein due to castration, which was reversed by testosterone injection. Immunohistochemistry showed a supranuclear localization of catD in the principal cells. The reaction was more intense in castrated animals, consistent with the immunoblotting results. Co-immunoprecipitation, demonstrated that secreted catD was increased and associated in part to PSAP. Immunohistochemistry with anti-PSAP-ab demonstrated that PSAP staining was not affected by castration. However, immunoblotting showed that castration decreased monomeric PSAP (70kDa) and increased oligomeric PSAP (250kDa) in the fluid. Similar to catD, PSAP showed a supranuclear staining in the principal cells that spread over the entire apical cytoplasm after castration. Both immunoblotting and immunohistochemistry with anti-sortilin ab indicated that the expression of this protein decreased with castration. This effect was reversed by testosterone replacement. Our results suggest that lower level of sortilin induced by castration increased PSAP oligomerization. We hypothesize that PSAP oligomers associate to catD increasing its secretion. In conclusion, testosterone may regulate the transport and secretion of catD creating an optimal environment for the sperm maturation.

POSTER 7

EFFECTS OF DNMT3L HAPLOINSUFFICIENCY ON THE DYNAMICS OF MALE GERM CELL DNA METHYLATION PATTERN ESTABLISHMENT

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DNA methylation patterns in male germ cells are initially acquired during prenatal development. While the majority of these patterns are established prior to birth, low resolution genome-wide studies have indicated that DNA methylation continues to be remodeled until the pachytene phase of spermatogenesis. Previous work has demonstrated that although mice haploinsufficient for the DNA methyltransferase-3-like (DNMT3L) enzyme have a delay in pre-natal DNA methylation acquisition, these patterns are comparable to those of wildtype littermates by postnatal day (PND) 6. Nonetheless, meiotic cells of *Dnmt3L+/-* males show chromatin abnormalities and the offspring exhibit an increased incidence of sex chromosome aneuploidy. In this study, we used next generation sequencing techniques to determine the dynamics of postnatal epigenetic pattern acquisition and determine the effects of *Dnmt3L* haploinsufficiency. Spermatogonia (n=4) from PND4 mice as well as mature sperm (n=3-5) were collected from both wildtype and *Dnmt3L+/-* males. Global DNA methylation analysis was undertaken using reduced representation bisulfite sequencing.

Results obtained demonstrated that the epigenetic patterning of germ cells is still evolving over the course of spermatogenesis, with 5398 100-base pair tiles differing in DNA methylation levels between day 4 spermatogonia and mature sperm. The majority of these were gains in methylation and the main regions undergoing pattern changes were within intergenic regions, exons and introns (2651, 1215 and 1202 tiles, respectively). Examination at day 4 revealed that DNA methylation defects were observed in spermatogonia due to *Dnmt3L* haploinsufficiency; the majority of these differences were hypomethylation (1965 of 2065 tiles changed; 97%). Methylation defects were still observed in mature sperm, with 827 tiles differing between genotypes.

Our results indicate that epigenetic patterning is still ongoing during early spermatogenesis. As well, DNA methylation patterns are still not resolved in the early postnatal germ cells in *Dnmt3L+/-* mice. These abnormalities may cause chromatin instability in these cells as they enter meiosis and may help explain reproductive defects in the adult mice.

POSTER 8

CHRONIC CYCLOPHOSPHAMIDE TREATMENT AFFECTS GENE EXPRESSION IN PACHYTENE SPERMATOCYTES AND ROUND SPERMATIDS

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Previous studies from our laboratory have shown that paternal exposure to cyclophosphamide, a chemotherapeutic agent and immunosuppressant, has detrimental effects on sperm quality and progeny outcome. How cyclophosphamide affects the developing germ cells and how they respond to this insult remain unresolved. The purpose of this study is to test the hypothesis that cyclophosphamide affects gene expression in pachytene spermatocytes and round spermatids. Adult Sprague-Dawley male rats were gavaged with cyclophosphamide (6 mg/kg) or saline, 6days/week for 4 weeks. Pachytene spermatocytes (n=5) and round spermatids (n=6) were collected by unit gravity sedimentation using the STA-PUT method. Total RNA was isolated and mRNA expression was profiled using whole genome gene expression microarrays. Data wasanalyzed with Genespring 12.0 and Pathway Studios software.

CPA treatment alters global gene expression in both pachytene spermatocytes and roundspermatids. Differential expression of transcripts coding for genes involved in the response toDNA damage and the regulation of cell death is observed in both cell types. However, the genesinvolved in these processes differ between the two cell types. CPA treatment alters theexpression of more genes involved in DNA damage in pachytene spermatocytes compared toround spermatids. Furthermore, altered transcripts in the pachytene spermatocytes are directlyinvolved in the apoptotic pathway.

These results suggest that chronic cyclophosphamide treatment results in different DNA damageand survival responses in pachytene spermatocytes and round spermatids. The altered ability ofthese cells to respond to DNA damage and survive may lead to damaged mature spermatozoa. These studies are supported by CIHR.

POSTER 9

FSH REGULATES INTRACELLULAR COMMUNICATION BETWEEN THE GROWING OOCYTE AND ITS FOLLICULAR MICROENVIRONMENT.

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In mammalian females, development of the oocyte depends critically on bidirectional communication with the granulosa cells (GCs) surrounding it. The mechanisms that regulate this communication, which depends on physical contact between the oocyte and GCs, have not been identified. We previously showed that mice which are unable to produce follicle-stimulating hormone (FSH), owing to a targeted mutation in the *Fshb* gene, produce oocytes with reduced developmental competence. As FSH targets GCs only, we hypothesized that FSH promotes oocyte development by modulating its communication with GCs. Using *Fshb-/-* mice and heterozygous controls, we found that during the late stage of folliculogenesis, *Fshb-/-* oocytes showed significantly reduced expression of the key cell adhesion and gap junction (GJ) components, *Cdh2* and *Gja4*. Since adherens and GJs between the oocyte and the GCs form at sites established by trans-zonal projections (TZPs), we next evaluated these structures in *Fshb-/-*. We found that in *Fshb-/-* complexes significantly fewer actin-rich TZPs projected to the oocytes. Since actin-rich TZPs are known to form contact sites for GJs, we next examined GJ coupling between the oocyte and the GCs, using a FLIP (fluorescence loss in photobleaching)-based protocol. Results showed that GJ communication between the oocyte and GCs was significantly impaired in *Fshb-/-* complexes. Thus, FSH promotes the expression of cadherins and connexins in the oocyte, increases the density of TZPs that link it to the GCs, and enhances GJ coupling between the two cell types. Our results identify FSH as the first extracellular factor shown to regulate communication between the developing oocyte and its somatic microenvironment.

POSTER 10

DEGRADATION OF MATERNAL TRANSCRIPTS IS INEFFICIENT *IN-VITRO*

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The final stage of oocyte development prior to fertilization, termed meiotic maturation, occurs *in vivo* in response to the gonadotropic signals that also trigger ovulation. Recently, oocyte maturation *in-vitro* has been introduced clinically, particularly for women who are at risk for ovarian hyperstimulation. However, for unknown reasons, oocytes matured *in-vitro* develop poorly as embryos. A key event of maturation is the degradation of many messenger RNAs (mRNA) that have accumulated during oocyte growth. Maternal transcript degradation is a prerequisite for the maternal-zygotic transition (MZT), yet the mechanisms controlling mRNA degradation are poorly understood. We compared the pattern of degradation of three mRNAs: *Orc6L, Gld and Slbp* during maturation *in-vivo* and *in-vitro*. Following maturation *in-vivo*, all three mRNAs were present at <10% of their quantity in immature oocytes. To induce maturation *in-vitro*, mice were injected with equine chorionic gonadotropin and immature oocytes were recovered 48 hr later and incubated in medium supplemented with 10ng/ml epidermal growth factor (EGF). After 22 hours, cumulus expansion had occurred, confirming the efficiency of the EGF. Oocytes that had reached metaphase II were analyzed. We observed no decline in the quantity of any of the three mRNAs examined. Similar results were observed when 10% fetal bovine serum was added to the maturation medium. These results indicate that commonly used *in vitro* conditions do not support degradation of maternal mRNAs which may underlie the poor developmental competence of *in vitro*-matured oocytes.

POSTER 11

MEIOTIC SILENCING DOES NOT AFFECT GENE EXPRESSION IN EMBRYOS SIRED BY HETEROZYGOUS ROBERTSONIAN TRANSLOCATION CARRIER

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Robertsonian (Rb) translocations in heterozygosis form trivalent structures in prophase which incompletely synapse at the centromeric regions. Failure of synapsis in prophase I of meiosis results in epigenetic silencing termed meiotic silencing of unsynapsed chromatin (MSUC) and abnormal chromosomal segregation. We studied MSUC in heterozygous carriers of one (Rb 8,12) and three (Rb 1,3; Rb 8,12; Rb 9,14) Robertsonian translocations. Previous work in our lab has shown a heterogeneity of the spermatocyte population with respect to MSUC with 10% of metaphase spermatocytes from single translocation carriers and 30% of three translocation carriers retaining MSUC marks at trivalents. The embryo epigenome undergoes major remodeling during preimplantation development. However, occasionally parental epigenetic marks resist reprogramming and, in principle, may influence embryo's phenotype. Here, we asked if MSUC marks are retained during gametogenesis and inherited by the offspring of translocation carriers causing a reduction of expression of genes associated with MSUC in embryos. If this hypothesis is correct, we expect to find significantly reduced expression of pericentromeric genes on chromosomes 8 and 12 in 10% or 30% of embryos from single or three translocation carriers, respectively.

To test our hypothesis, we first characterized the viability of offspring from translocation carrier fathers at birth, at 9.5 and 7.5 days post coitum (dpc). We observed a reduction of fertility in carriers of three translocations (but not single translocation) compared to wild type controls. Carriers of three translocations had an average litter size of 3.5 pups (n=10), whereas wild type controls had 8.7 pups (n=24) per litter. To determine if loss of embryos occurred post-implantation, embryos were collected at 7.5 and 9.5 dpc and found that embryos were lost from 7.5 dpc to birth. To maximize the chances of detecting the effect of MSUC we conducted expression studies in 9.5 dpc embryos. We compared expression levels in 28 9.5 dpc embryos from an intercross between single translocation carriers and 20 control embryos. Since Rb translocations cause chromosomal segregation errors, we expect to detect trisomies in translocation-associated chromosomes, but not monosomies as embryos with monosomies do not implant. Hence, all embryos with reduced gene expression at pericentromeric regions will be likely those with inherited MSUC marks.

Expression analysis detected embryos with increased expression of genes (trisomies) for chromosomes 12 and 8, as well as reduction of expression. However, embryos with reduced expression were found among controls too. Possible explanations for these findings include: 1) we cannot reliably detect MSUC effects due to inter-individual variation; or 2) MSUC-associated marks are not transmitted to offspring. Further studies are necessary to determine which of these scenarios is the correct one.

POSTER 12

CHARACTERIZATION OF SEC23A AND MAN1B1 EXPRESSION AND FUNCTION IN WILDTYPE AND MUTANT FIBROBLASTS

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SEC23A and *MAN1B1* are essential genes involved in protein secretory pathway. SEC23A is an essential component of COPII-coated vesicles that transport secretory proteins from the endoplasmic reticulum (ER) to the Golgi complex and Alpha 1,2-mannosidase (MAN1B1) is an essential enzyme required for targeting proteins into the Endoplasmic-reticulum-associated protein degradation pathway. Mutation in *SEC23A* is associated with craniolenticulosutural dysplasia (CLSD) whereas mutation in MAN1B1 is associated with nonsyndromic autosomal-recessive intellectual disability (NS-ARID) and congenital disorders of glycosylation (CDG)-II. Mutations in *SEC23A* or MAN1B1 are extremely rare and have only been reported in a handful of patients. Our group identified a novel missense mutation in *SEC23A* c.1200G>C (p.M400I) and a previously identified mutation in *MAN1B1* c.1000C>T (p.R334C) using whole exome sequencing in two boys suffering from macrocephaly, moderate global developmental delay, mild dysmorphic features, obesity, hypertelorism, and intellectual disability. The parents are first cousins. In this poster we show a significant decrease in MAN1B1 and normal level of SEC23A using westen blot analysis in mutant fibroblasts. We observed in mutant fibroblasts distended ER membranes; compacted and fewer Golgi bodies; and reduced numbers of COPII vesicles through Transmission electron microscopy. We determine procollagen1, a previously identified cargo of SEC23A was properly localized to the ER but abnormally secreted in mutant fibroblasts. However, we did not observe activation of the unfolded response pathway. The phenotype of our patients and our molecular analysis suggest a genetic interaction between these two genes.

POSTER 13

DECIPHERING THE ROLE OF THE NODAL SIGNALING PATHWAY IN PRETERM BIRTH

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Preterm birth, defined as delivery before 37 weeks of gestation, is affecting up to 12-13% of pregnancies. Despite the prevalence and severity of premature delivery, the mechanisms and causes that underlie spontaneous and idiopathic preterm birth are still unknown. Our lab has been studying the role of Nodal signaling pathway during pregnancy. Nodal is a morphogen belonging to the transforming growth factor-beta (TGF-) superfamily. Nodal has been shown to play critical functions during embryonic development and our lab has recently shown that it is also implicated in mammalian reproduction. We have demonstrated that Nodal signaling active in uterus during early pregnancy is required both for embryo implantation and for the timing of parturition. We have generated a uterine specific deletion using a progesterone receptor-Cre (PR-Cre) and observed a 75% reduction in the rate of pregnancy after mating. Furthermore, the 25% of Nodal mutant females that did become pregnant most of them gave birth two days prior to term. Interestingly, our preliminarily data show that even Nodal heterozygous mice have a predisposition to preterm birth as several females have given birth on day 17.5. Based on our results and the fact that Nodal expression is conserved in the human endometrium, we hypothesized that Nodal plays a crucial role in the timing of parturition and that mutations within the Nodal gene or deregulated Nodal activity in the uterus leads to an increase in the incidence of preterm birth in human. To test this hypothesis we will sequence the Nodal gene and determine the level of uterine expression in mothers that have given birth preterm and compare this to the sequence and levels of expression in mothers who had term pregnancies to determine if there is a correlation between Nodal expression/function and preterm birth.

Project funded by the March of Dimes Grant #21-FY14-130

POSTER 14

IS TMED2 ESSENTIAL IN THE CHORION FOR NORMAL INTERACTION BETWEEN THE ALLANTOIS AND THE CHORION IN MICE?

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During vesicular transport between the endoplasmic reticulum and the Golgi, members of the transmembrane emp24 domain (TMED) protein family form hetero-oligomeric complexes that facilitate protein cargo transportation and secretion. In our laboratory, we are studying the function of one member of the TMED protein family, TMED2, in mouse placental labyrinth development. Formation of the mouse labyrinth layer requires proper interactions between two extraembryonic tissues, the allantois and the chorion; and is essential for nutrition, waste, as well as hormone exchange between fetal and maternal circulation. We have shown that *Tmed2* is expressed in both allantois and chorion and is required for normal labyrinth layer formation. In *Tmed2* mutants, expression of syncytiotrophoblast cell marker, *Gcm1* is reduced. We hypothesized that TMED2 is essential in the chorion for normal interaction between the allantois and chorion- a critical step in placental labyrinth layer development. To test this hypothesis, we have generated an ex-vivo allantois and chorion recombination model. In our model, we recapitulated the early events of labyrinth layer development: chorioallantoic attachment, fusion of the mesothelium and allantois, and chorionic trophoblast differentiation. We used in situ hybridization (ISH) and immunohistochemistry to confirm the chorioallantoic attachment event and to monitor development of labyrinth layer in the chimeric explants. Our data showed that the chorioallantoic attachment is required for maintaining expression of syncytiotrophoblast cell marker, *Gcm1*. Our data suggest a previously unknown requirement for chorioallantoic attachment for maintenance of the spongiotrophoblast cell marker, *Tpbpa* in *ex-vivo* cultures. In our preliminary results, we used combinations of wild type and *Tmed2* null chorion and allantois in these ex-vivo cultures to determine which tissue TMED2 is required in for normal chorioallantoic attachment. The histological Hemotoxylin and Eosin stain showed successful chorioallantoic attachment between *Tmed2* null allantois and wildtype chorion as well as *Tmed2* null allantois and *Tmed2* null chorion recombinants. Currently, ISH is performed to determine the expression pattern of different placental markers such as *Gcm1*, and *Tpbpa* in those mutant configurations.

POSTER 15

ROLE OF ATAD3 IN THE HORMONE-INDUCED ER-MITOCHONDRIAL ORGANIZATION IN HORMONE-INDUCED LEYDIG CELL STEROIDOGENESIS

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Leydig cell steroid formation is a multi-step process initiated in mitochondria, using cholesterol coming from intracellular stores, and finalized in the endoplasmic reticulum (ER). The AAA+ATPase ATAD3 is anchored in the inner mitochondrial membrane and enriched at outer-inner mitochondrial membrane contact sites. The long isoform of ATAD3 possess an N terminus domain with 50 amino acids able to drive the insertion of the protein back into the outer membrane. We hypothesized that the physical association between mitochondria and ER, named mitochondria-associated membranes (MAMs), can regulate hormone-stimulated steroidogenesis. Using MA-10 Leydig cells and electron and confocal microscopy, we observed a significant increase of MAM formation upon hGC stimulation. MAMs were isolated and characterized. We observed an enrichment of the long isoform in MAMs. Silencing ATAD3 resulted in reduced ability to form pregnenolone and progesterone in response to hCG, but not 22-R hydroxycholesterol, confirming the role of ATAD3 at the level of cholesterol delivery into mitochondria. Since progesterone is made mainly in the ER, and a profound modification of the mitochondrial inner structure was observed, we suggest that ATAD3 functions not only as a bridge between outer-inner mitochondrial membranes but also might be involved in the organization of MAMs. MAMs could allow the transfer of cholesterol into mitochondria for steroidogenesis. Deletion of the anchoring ATAD3 N-terminus blocked the hormone-induced steroid formation further supporting ATAD3's role in MAM formation. Taken together, these results suggest a role of ATAD3 as a scaffold protein in the regulation of ER-mitochondria communications, crucial for optimal hormone-stimulated steroidogenesis.

POSTER 16

LONG TERM EFFECTS OF LOW DOSE PRENATAL EXPOSURE TO ENDOCRINE DISRUPTORS ON MALE REPRODUCTION AND DEVELOPMENT

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Environmental exposures to endocrine disruptors (EDs) are thought to contribute to idiopathic increases in male reproductive abnormalities. Although humans are exposed to a myriad of EDs from conception through adulthood, few studies have evaluated the effects of combined exposures, while animal studies often used doses exceeding environmental levels. We hypothesized that prenatal exposure to combined low dose of the phytoestrogen genistein and the plasticizer di-(2-ethylhexyl) phthalate (DEHP) might pose a greater risk to male reproduction than individual compounds.

Pregnant Sprague Dawley dams were gavaged from gestational day 14 to birth with either corn oil, genistein, DEHP or their mixture at 10 mg/kg/day, a dose at which either compound does not cause conspicuous long term effects. Adult serum testosterone levels were unchanged. However, histological analysis revealed increased interstitial fibrosis in testes of ED-exposed adult offspring, suggestive of premature aging. Quantification of elongated spermatids and quantitative PCR analysis (qPCR) of germ cell markers indicated adult germ cell alterations in ED-exposed rats.

Gene expression arrays identified 83 up-regulated and 171 down-regulated genes ($p<0.05$) in testicular RNA from ED-treated relative to control rats, including genes with explicit ties to male reproduction, such as FOXA3, PDGFRα and HSD3β1, suggesting disrupted testis development and function. These changes were further validated by qPCR.

These results show that prenatal exposure to a mixture of unrelated EDs at environmentally relevant doses permanently disrupt testicular gene expression profiles and histology in a manner different from individual compounds, highlighting a need for more realistic and comprehensive analysis of endocrine disrupting chemicals.

This work was supported by funds from the Research Institute and an award from the Royal Victoria Hospital foundation at the McGill University Health Center and a CIHR Grant (# MOP-312268) to MC and a NSERC scholarship to SJ.

POSTER 17

THE HISTONE H3 DEMETHYLASE, *KDM1A* IS ESSENTIAL FOR THE DIFFERENTIATION OF SPERMATOGONIA AND THE SURVIVAL OF SPERMATOGONIAL STEM CELLS

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Spermatogenesis is a highly complex cell differentiation process fueled by spermatogonial stem cells (SSCs). The progression from a spermatogonial stem cell to a differentiating cell involves gene expression changes that are under epigenetic control. KDM1A is a histone demethylase that removes gene-activating methylation on histone H3 at lysine 4 (K4). We had previously observed that KDM1A was present in SSCs, hence we hypothesized that this protein serves in the epigenetic regulation of SSCs biology. To determine the function of *Kdm1a* in SSCs we generated mice with a conditional knockout of *Kdm1a* (cKO) specifically in spermatogonia. Analysis of the cKO revealed that *Kdm1a* is essential for spermatogenesis, as adult cKO males were sterile and lacked germ cells. Testes were collected from cKOs at post-natal days (PND) corresponding to the appearance of spermatogonia (PND6) and meiotic cells (PND10). At PND6, spermatogonia were still present in the cKOs; however, at PND10 very few cells with an abnormal morphology were observed in place of preleptotene spermatocytes. Moreover from PND10 to 21 the number of spermatogonia in the cKO testes decreased dramatically with no germ cell remaining at PND21. We then used RNA-sequencing to examine how *Kdm1a* loss alters the gene expression profile of isolated PND6 spermatogonia. We observed that 1206 genes had an altered expression in the cKOs, a majority of which was upregulated. Interestingly the expression of the undifferentiated spermatogonia marker *Plzf* was decreased in the absence of *Kdm1a* as was that of genes essential for spermatogonial stem cell maintenance such as *Oct6*, *Sall4*, *Lin28a* and *Nanos2/3* and genes implicated in differentiation such as *Kit* and *Neurog3*. These results suggest that *Kdm1a* is a master epigenetic regulator of spermatogonia required for SSCs survival and spermatogonia differentiation.

POSTER 18

FOLLICULOGENESIS IS AFFECTED BY AN ENVIRONMENTALLY RELEVANT BROMINATED FLAME RETARDANT MIXTURE IN SPRAGUE-DAWLEY RATS

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Chronic exposure to toxic chemicals in the environment may contribute to the recent increase in infertility. Brominated flame retardants (BFRs) may act as endocrine disrupters; Pregnancy failure has been associated with BFRs in human follicular fluid, raising serious questions regarding their impact on female reproductive health. Our goal was to assess the effects of exposure to an environmentally relevant BFR mixture on female reproductive health using a rat model. A BFR mixture was formulated to mimic the relative congener levels in house dust, the major source of human exposure. Adult female Sprague-Dawley rats received this BFR mixture in the diet (0, 0.06, 20 or 60 mg/kg/day) from 2-3 weeks before mating until gestation day (GD) 20. Reproductive parameters, such as mating, fertility indices and litter size, were analyzed. Levels of estradiol (E2), follicle-stimulating hormone (FSH), luteinizing hormone (LH) and anti Mullerian hormone (AMH) were measured in dam serum samples. Folliculogenesis was evaluated by counting primordial, primary, secondary and antral follicles and, measuring follicle diameters and areas in sections from five ovaries per experimental group. BFR exposure had no significant effect on estrous cyclicity, reproductive success, or serum hormones in the dams. However, BFR exposure induced a significant dose-dependent increase in the numbers of primary ($r=0.462$; $p=0.0456$) and antral follicles ($r=0.501$; $p=0.0243$). At the low and high doses of the BFR mixture, antral follicles were enlarged, as reflected by a significant increase in both diameter ($p=0.002$) and area ($p=0.01$). Exposure to an environmentally relevant BFR mixture affects folliculogenesis in the rat. *Supported by CIHR grant RHF100625, FRQS (PL), REDIH (PL) and RQR- CREATE (RB).*

POSTER 19

ENHANCED FERTILITY IN FEMALE MICE WITH A GONADOTROPE-SPECIFIC DELETION OF THE INHIBIN CO-RECEPTOR TGFBR3 (BETAGLYCAN)

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FSH is an essential regulator of ovarian follicle development and fertility in females and of quantitatively normal spermatogenesis in males. FSH synthesis is stimulated by intra-pituitary activins and is suppressed by gonadal inhibins. Activins stimulate transcription of the FSH β subunit gene (*Fshb*) in gonadotrope cells. Inhibins, in contrast, impair FSH production via competitive antagonism of activin signaling. That is, inhibins bind the activin type II receptor, ACVR2, with picomolar affinity in the presence of the co-receptor TGFBR3 (also known as betaglycan), blocking autocrine/paracrine activin signaling. The current model of inhibin action via TGFBR3 is based on *in vitro* observations. *Tgfbr3*-deficient mice die during embryonic development, precluding their use for in vivo assessment of TGFBR3 function. We therefore employed a conditional knockout approach (Cre/lox) to investigate the necessity for TGFBR3 in inhibin action and FSH synthesis in gonadotropes *in vivo*. First, we generated a conditional (floxed) *Tgfbr3* allele by gene targeting in murine embryonic stem cells. Cre-mediated recombination deletes exon 2, generating a loss of function allele. Indeed, mice homozygous for the globally recombined *Tgfbr3* allele died during embryonic development. Next, we assessed TGFBR3's role in inhibin action in primary pituitary cultures of Tgfbr3flox/flox mice. Inhibin A potently and dose-dependently inhibited FSH secretion and *Fshb* mRNA expression in cultures transduced with a control (GFP-expressing) adenovirus. In contrast, inhibin A action was abrogated in cells transduced with a Cre-expressing adenovirus. Finally, we selectively ablated *Tgfbr3* in gonadotropes by crossing *Tgfbr3*$^{flox/flox}$ and GnrhrIRES-Cre (GRIC) mice (hereafter, T3cKO). We confirmed the selective reduction of TGFBR3 protein expression in gonadotropes of T3cKO mice by immunofluorescence. Whereas T3cKO males had normal reproductive organ weights and serum FSH, their pituitary *Fshb* mRNA levels were significantly increased. T3cKO females were super-fertile, producing approximately two more pups per litter than controls (7.5±0.5 vs. 9.6±0.5) over a 6 month breeding trial. T3cKO ovaries contained a greater number of corpora lutea than controls (4.7±0.5 vs. 6.6±0.9 per ovary). We are currently investigating FSH levels and antral follicle numbers in females. Collectively, these data suggest that inhibins act via TGFBR3 in gonadotropes to suppress FSH synthesis *in vivo*.

POSTER 20

EFFECTS OF FOUR CHEMOTHERAPEUTIC AGENTS, BLEOMYCIN, ETOPOSIDE, CISPLATIN AND CYCLOPHOSPHAMIDE, ON DNA DAMAGE AND TELOMERES IN A MOUSE SPERMATOGONIAL CELL LINE.

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Treatment with chemotherapeutics agents may induce persistent DNA damage in male germ cells with the possibility of long term consequences on fertility and progeny outcome. Telomeres, specialized structures at the physical ends of chromosomes, play an important role in the maintenance of genetic stability and in the response of somatic cells to anticancer drugs. Our objective was to test the hypothesis that exposure to bleomycin, etoposide, or cisplatin (the drugs used to treat testicular cancer) or cyclophosphamide (an anticancer agent and immunosuppressant) targets telomeres in the male germ line. C18-4 spermatogonial cells were exposed to bleomycin, etoposide, cisplatin or 4-hydroperoxycyclophosphamide (4OOH-CPA, a pre-activated analog of cyclophosphamide). All four anticancer drugs induced a significant increase in DNA damage in C18-4 cells, as assessed by γH2AX immunofluorescence. Interestingly, the γH2AX signal was localized to telomeres after treatment with bleomycin, cisplatin, and 4OOH-CPA, but not etoposide. Mean telomere lengths, the intensity of the telomere fluorescence in situ hybridization (FISH) signal, telomerase activity, and the expression of the telomerase enzyme mRNAs components, *Tert* and *Terc*, were reduced by exposure to cisplatin and 4OOH-CPA, but not by bleomycin or etoposide. Thus, while all four anticancer drugs induced DNA damage in this spermatogonial cell line, telomere were not specifically affected by etoposide and only the two alkylating agents, cisplatin and 4OOH-CPA, induced telomere dysfunction. This telomere dysfunction may contribute to infertility and developmental defects in the offspring.

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POSTER 21

UNRAVELLING THE MYSTERY OF BASAL CELLS: ISOLATION AND CHARACTERIZATION OF RAT EPIDIDYMAL BASAL CELLS

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Epididymal basal cells are localized to the basal compartment of the epithelium. They have thin processes that extend along the base of the epithelium and that can extend apically. Little information exists on the function of epididymal basal cells. Studies suggest that these may be part of the immune system and regulate other epithelial cells and the blood-epididymis barrier. The present objective was to develop a reproducible protocol to isolate a relatively pure fraction of epididymal basal cells in order to characterize their function. Immunolocalization indicated that integrin-α6 was localized to basal cells in all regions of the rat epididymis. Epididymides from 42 day old rat (absence of sperm) were enzymatically digested and cells isolated using magnetic microbeads coupled with the integrin-α6 antibody. Immunofluorescence using cytokeratin 5 revealed a 90% enrichment of basal cells. Flow cytometry with another basal cell marker, cyclooxygenase 1 (Cox1), confirmed these results. Total cellular RNA isolated from the enriched basal cell fraction was subjected to RT-PCR using specific markers of basal, principal, dendritic, and clear cells. Results indicated that the isolated basal cell fraction was enriched for basal cells while markers of other cell types were lower. Electron microscopy of isolated cells revealed morphological characteristics of basal cells. This novel approach represents a unique method to reproducibly isolate epididymal basal cells in order to understand the role and function of these cells. Supported by CIHR and FQRNT.

POSTER 22

UNRAVELLING THE MYSTERY OF BASAL CELLS: ROLE OF TRICELLULIN AND THE BLOOD-EPIDIDYMIS BARRIER

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Tricellulin is a tight junction protein implicated in tricellular junctions. Studies suggest that basal cells are implicated in the blood-epididymis barrier. While basal cells express Claudin1 (CLDN1), there is no information regarding the potential architecture or regulation of basal-principal cell tight junctions. The present objective was to explore the expression and localization of tricellulin in rat epididymis in relation to basal-principal cell interactions. Tricellulin levels were similar in all segments of the adult epididymis and localized to the area of the BEB. Postnatal development studies showed that tricellulin levels increased with age and its localization varied. At day 14, tricellulin was cytoplasmic while at day 21 it began to localize to the lateral margins of the plasma membrane which became more pronounce at 42 and 56 days. Co-localization with occludin indicated that in the initial segment, occludin and tricellulin did not co-localize but rather localize to different areas of the junctional complex; in other regions both proteins co-localized to the BEB. Co-localization of tricellulin and KRT5 (cytokeratin5), a marker of basal cells, indicated that these co-localized in areas where basal cell projections occur suggesting tripartite junctions between basal and principal cells. Tricellulin knock-down using siRNA and epididymal RCE cells resulted in decreased transepithelial resistance. Furthermore, levels of occludin, CLDN3, and CLDN1 were decreased while ZO-1 and E-cadherin levels were unchanged. Results indicate that tricellulin is implicated in tight junctions of the BEB and that in the corpus and cauda it is implicated in basal-principal cell interactions. Supported by CIHR.

POSTER 23

THE EFFECT OF PATERNAL AGING ON SPERM NUCLEUS AND REPRODUCTIVE SUCCESS OF Prdx6-NULL MALES

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Increasing paternal age in developed countries over the past 40 years raised the question of how aging affects reproductive success. Spermatozoa from infertile men have low levels of peroxiredoxin 6 (PRDX6) along with high levels of DNA damage and impaired motility. We hypothesize that the absence of PRDX6 impairs male reproduction, which is worsen with age. Our objective was to investigate the impact of paternal aging on the integrity of sperm nucleus and reproductive success of Prdx6-null males. Two-, 8- or 20-month-old Prdx6-null males and their wild type controls were used to determine DNA fragmentation levels (DFI) using Sperm Chromatin Structure Assay, DNA compaction by high DNA stainability (HDS) and level of free thiols and of protamination by monobromobimane (mBBr) or chromomycin A3 (CMA3) labeling, respectively by flow cytometry, DNA oxidation by 8-hydroxy-2' -deoxyguanosine (8-OHdG) levels determined by immunocytochemistry along with sperm motility, cytoplasmic droplet retention (CDR) and number of pups or litters/male. Absence of PRDX6 caused age-dependent decrease of sperm motility and increase of CDR compared to wild type controls. Prdx6-null mice showed increased DFI, HDS, CMA3 and mBBr-labeling and increased DNA oxidation compared to wild type controls. At 8 and 20 months of age, PRDX6-null males showed low motility, increased CDR, DNA fragmentation and oxidation and lower DNA compaction (HDS) compared to young controls. Reproductive outcomes were significantly lower in Prdx6-null males compared to controls and worsen with age. Advanced paternal age affects sperm chromatin integrity, epididymal maturation and fertility more severely in the absence of PRDX6. This suggests a protective role for PRDX6 in age-associated decline in the sperm quality and potential biomarker for male fertility in assisted reproductive technologies. Supported by CIHR, FRQS and RQR.

POSTER 24

THE ROLE OF RSK3 IN GRANULOSA CELLS OF OVULATING FOLLICLES IN MICE

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It is established that the extracellular signal-regulated kinase ERK1/2 plays an indispensable role in the preovulatory LH signaling in granulosa cells. Therefore, we hypothesized that p90 ribosomal s6 kinases (RSKs), which act as downstream mediators of ERK1/2, contribute to LH signaling during ovulation. Quantitative PCR analyses, using C57BL/6NCrl mice, revealed that the ovulatory dose of human chorionic gonadotropin (hCG) increased mRNA levels of *RSK3* by 4h post-treatment in granulosa cells of ovulating follicles. We examined the role of *RSK3* using recently developed KO (*RSK3-/-)* mice. Growth rate in *RSK3-/-* mice was normal, as evidenced by their similar body weights to the age matched wild type (*RSK3+/+)* mice. However, we observed sub-fertility with reduced litter size ($P < 0.05$) and ovulation ($P<0.01$) compared to *RSK3+/+* mice, possibly linked to ovarian dysfunction. Further gene expression analysis revealed transcript abundance of A disintegrin and metalloproteinase with thrombospondin motifs 1 (*Adamts1*) was higher in *RSK3-/-* granulosa cells ($P < 0.05$), while that of matrix metalloproteinases (*Mmp 11* and *23*) was similar between the two genotypes ($P > 0.05$). However, the mRNA abundance of the tissue inhibitor metalloproteinase 1 (*Timp1*) and serine peptidase inhibitor clade B6a (*Serpinb6a*) was also higher in *RSK3-/-* than WT granulosa cells. In accordance with increased expression of *Timp1*, there was a significant increase in signal transducer and activator of transcription-3 (*Stat3*) ($P<0.05$). These results indicate an imbalance in the activity of the proteases and their inhibitors in ovulating follicles of *RSK3-/-* mice. Therefore, we concluded that *RSK3* signaling regulates ovulation through maintaining a tight balance between extracellular matrix proteases and their inhibitors during ovulation.

POSTER 25

NEPHRIC DUCT FORMATION REQUIRES THE PRESENCE OF CLAUDIN FAMILY MEMBERS.

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During kidney formation, polarized epithelial cells are required in the first events of tubulogenesis. Epithelial cells are linked at their apical side by tight junctions, which act as fences that maintain the apical localization of proteins and as barriers that regulate the passage of ions through the paracellular space. Claudin family members are one of the main components of the tight junctions and are important players in the formation and maintenance of those junctions. They have 4 transmembrane domains, 2 extracellular loops a cytoplasmic N-terminus and C-terminus, the latest linking them to the actin-cytoskeleton via adaptor proteins. Their first extracellular loop is responsible for the ion selectivity of the tight junction and their second extracellular loop is responsible for homotypic and heterotypic interactions between claudins. Some claudins have a *Clostridium perfringens* enterotoxin (CPE) binding site on their second extracellular loop. By using the non-toxic C-terminal portion of *Clostridium perfringens* enterotoxin (cCPE) multiple cCPE-sensitive claudins can be removed simultaneously from tight junctions. Claudin-1, -3 and -4 are expressed in the chick nephric duct, Claudin-3 and -4 being cCPE sensitive. cCPE-soaked beads were implanted in the intermediate mesoderm on the right side of the embryo prior to nephric duct formation. Whole mount *in situ* hybridization analysis of *Lim1* expression, which is expressed throughout the nephric duct, revealed that cCPE inhibited nephric duct formation. The effects of cCPE treatment on epithelialization of the cells that form the nephric duct, apical-basal polarity of cells within the duct and cell morphology are being analyzed.

POSTER 26

CYTOPLASMIC DEADENYLASE CNOT6 LOCALIZES TO CORTICAL FOCI IN MURINE OOCYTES AND PARTICIPATES IN THE DEADENYALTION OF SPECIFIC MATERNAL TRANSCRIPTS

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Transcription in growing oocytes is very active, accumulating large amounts of mRNA. Some of these transcripts however are kept in a quiescent state and recruited when transcription becomes undetectable after growth, during maturation and the initial steps of embryogenesis. This developmentally regulated silencing of maternal mRNAs results from the deadenylation of the poly (A) tail located at the 3'-end of the mRNA. However, the mechanism of deadenylation remains uncharacterized. We first identified in murine oocytes, the expression of *Cnot6*, encoding an enzymatic subunit of the major cytoplasmic deadenylase, CCR4-NOT. Immunoblot analysis revealed that the quantity of CNOT6 protein decreased relative to oocyte size (ie, concentration) during oocyte growth. Immunofluorescent assay of oocytes at different stages of growth pinpoint the localization of CNOT6 to cytoplasmic foci that aggregate at the cortex and that are also positive for p-body markers GW182 and LSM14A. Another P-body marker, DCP1A, shows a similar cortical pattern. A *Cnot6* knock-down using a *Cnot6*-specific siRNA, disrupts the cortical ring of CNOT6 foci and cortical fluorescence intensity significantly decreases, confirming the specificity of the antibody. Depletion of *Cnot6* also prevents the deadenylation of the maternal transcript *Slbp* during maturation as assessed by RL-PAT. We propose that in murine oocytes, CNOT6 is recruited to cytoplasmic ribonucleoprotein complexes that specialize in mRNA processing, where it deadenylates specific maternal transcripts.

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COMBINED EFFECTS OF DNA METHYLTRANSFERASE 1O (DNMT1O) DEFICIENCY, SUPEROVULATION ON EPIGENETIC PATTERNS IN THE OFFSPRING

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Assisted reproductive technologies (ARTs) comprise several methods used to bypass infertility and achieve pregnancy. These technologies have been linked with an increased incidence of growth and genomic imprinting disorders in children, previously shown to be the result of aberrant DNA methylation. DNA methylation, a well-characterized epigenetic modification, is mediated by DNA methyltransferases (DNMTs). Oocyte-specific DNMT1o is responsible for imprint maintenance at the 8-cell stage of pre-implantation development. In offspring of female *Dnmt1o*-knockout mice, embryos are developmentally delayed and morphologically abnormal.

Our aim is to assess the combined effects of superovulation and maternal DNMT1o-deficiency, representative of poor quality or aged oocytes, on offspring DNA methylation and development. We hypothesize that reduced expression of DNMT1o will increase susceptibility to imprinting and genome-wide methylation abnormalities, exacerbated by ARTs. Preimplantation embryos collected from superovulated (5.0IU PMSG/hCG) 129/Sv *Dnmt1o*+/+ and *Dnmt1o*+/- females (2-6 months; mated with 129/Sv males) were transferred non-surgically into uteri of pseudo-pregnant CD1 recipients.

Forty-three embryos have been collected: 24 from *Dnmt1o*+/+ and 19 from *Dnmt1o*+/- superovulated donor females. The occurrence of developmental delays and morphological abnormalities was greater in embryos derived from DNMT1o-deficient females compared to control (42 and 8%, respectively). Additionally, we observed a higher male to female ratio in the heterozygote group than in the control (increased from 50 to 73%). DNMT1o deficiency has no apparent effect on DNA methylation at imprinted *H19* and *Snrpn* in female embryo and placenta as assessed by pyrosequencing. These preliminary results suggest that DNMT1o deficiency plays a key role in mediating poor embryonic outcome and suggests that female embryos may be more sensitive to this deficiency than males. (Funded by CIHR)

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Centre for the Study of/Centre d'études sur la Reproduction

HUMAN REPRODUCTION AND DEVELOPMENT

Breakthroughs in reproduction and development

Research Day of the Centre for the Study of Reproduction (CSR) at McGill
& the Human Reproduction and Development Axis of the RI-MUHC

Wednesday, May 7, 2014

EVENT EVALUATION SURVEY

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

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

The registration process	1	2	3	4	5
The guest speakers	1	2	3	4	5
The oral presentations	1	2	3	4	5
The poster presentations	1	2	3	4	5
The content of the sessions	1	2	3	4	5
The length of the sessions	1	2	3	4	5
The facility	1	2	3	4	5
The refreshments	1	2	3	4	5
Overall	1	2	3	4	5

Comments/Suggestions:

Centre for the Study of/Centre d'études sur la Reproduction

HUMAN REPRODUCTION AND DEVELOPMENT

Nouvelles avancées en reproduction et développement

Journée de recherche du Centre d'études sur la reproduction (CER) à McGill
et l'axe de la reproduction humaine et du développement de l'IR-CUSM

Mercredi 7 mai 2014

FORMULAIRE D'ÉVALUATION

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

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

Le processus d'inscription	1	2	3	4	5
Les présentateurs invités	1	2	3	4	5
Les présentations orales	1	2	3	4	5
La présentation des affiches	1	2	3	4	5
Le contenu des sessions	1	2	3	4	5
La durée des sessions	1	2	3	4	5
L'emplacement	1	2	3	4	5
La nourriture et les breuvages	1	2	3	4	5
En général	1	2	3	4	5

Commentaires/Suggestions: