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 4, 2011

McGill New Residence Hall
3625 avenue du Parc in Montreal
Montreal, Quebec

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

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To register: Fill in the registration form and return by email to csr-cer@mcgill.ca
or by fax to Aliea Jamal at 514-398-7120

8:00 AM — Registration and coffee / Poster set-up

8:45 — Opening remarks: **Dr. Bernard Robaire**

9:00 — **Dr. Haifan Lin, Professor of Cell Biology and Genetics, Director of Yale University Stem Cell Center**, "*A Novel Small RNA-Mediated Epigenetic Mechanism Related to Stem Cells*" - introduced by Bernard Robaire

9:45-10:30 — Oral presentations (Chairs: **Lisanne Grenier and Cristian O'Flaherty**)

O-01. **Nathalie Bédard, Division of Experimental Medicine, Department of Medicine, McGill University.** "*Mice Lacking the USP2 Deubiquitinating Enzyme have Severe Male Sub-Fertility Associated with Defects in Fertilization and Sperm Motility*"

O-02. **Ava Schlisser, Department of Pharmacology and Therapeutics, McGill University.** "*Hydroxyurea-Induced Caudal Malformations in Mice are Enhanced by Deprenyl*"

O-03. **Genevieve Plante, Department of Immunology-Oncology, University of Montreal** "*Binding and Functional Characteristics of Recombinant Murine Binder of Sperm (BSP) Proteins*"

10:30-11:45 — *Health break / Poster Session*

P-01. France-Helene Paradis, Department of Pharmacology and Therapeutics, McGill University "*Effects of Valproic acid and Valpromide on limb morphology and gene expression during development Correlate with their Histone Deacetylase InhIbition Activity*"

P-02. Gupreet Manku, PhD student at the McGill Department of Pharmacology and Therapeutics, "*From gonocyte differentiation to testicular tumors: where is the link?*"

P-03. Tania Morielli, MSc student at the McGill Department of McGill Urology Research Laboratory, "*Protein Tyrosine Nitration is Modulated by Reactive Oxygen Species and Differentially Localized in Human Spermatozoa*"

P-04. Marie-France Lusignan, PhD student at the University of Montreal Faculty of Medicine, "*Studies of the interaction between Binder of SPerm1 (BSP1) and egg yolk LDL*"
P-05. Halim Khairallah, PhD student in the McGill Department of Human Genetics and Pediatrics, "*The Role of Claudins in Kidney Morphogenesis*"
P-06. Oli Sarkar, Postdoctoral Fellow in the McGill Faculty of Medicine, "*Is v1-pdgfrβ Involved in the Progression from Proliferation to Differentiation in Testicular Neonatal Germ Cell?*"
P-07. Andrew Midzak, Postdoctoral Fellow in the McGill Department of Medicine, "*Structure-function Analysis of 5-androsten-3β,17,19-triol, a Novel Ligand for the Mitochondrial Translocator Protein, and Related Steroids which Control Steroidogenesis*"
P-08. Ahmed Barry, PhD student at the University of Montreal Department of Pathology and Cell Biology, "*Effet du bfgf sur l'expression et la phosphorylation de la connexine 43 des gap junctions dans les cellules folliculostellaires de l'anté-hypophyse ; Étude des voies de signalisation*"
P-09. Dora Siontas, PhD student in the McGill Department of Experimental Medicine, "*The Identification of Proteins That Interact with the N-Terminal Domain of PITX2C*"
P-10. Craig Park, PhD Student in the McGill Department of Experimental Medicine, "*Uterine-specific Nodal Deletion Disrupts Placentation and Contributes to Preterm Birth in Mice***"**
P-11. Sathvika Sanjiv Jagannathan, MSc student in the McGill Department of Medicine, "*Visualization of the Dynamic Formation of a Mitochondrial Cholesterol Transport Complex by Förster Resonance Energy Transfer Confocal Microscopy*"
P-12. Qin Yang, Research Assistant in the McGill Department of Obstetrics and Gynecology, "*Searching for Elements That control MRNA Translation in the Oocyte*"
P-13. Amanda Baumholtz, PhD student in the McGill Department of Human Genetics and Pediatrics, "*Manipulating Claudin Expression in the Epiblast***"**
P-14. Christopher Garcia, PhD student at the Univerity of Montreal Department of Pathology and Cell Biology, "*Connexins 46 and 50 in the Folliculostellate Cells of the Anterior Pituitary: Localization in the TTT/GF Cell Line, and Expression Dynamics in Response to BFGF and During the Mink Reproductive Cycle*"
P-15. Dyananda Siddappa, PhD student in the McGill Department of Animal Science, "*Mtor Activity in the Mouse Ovary*"
P-16. Tina Djogo, MSc student in the McGill Department of Obstetrics and Gynecology, "*WNT Protein Signaling and its Role During the Process of Implantation*"
P-17. Evelyn Llerena, MSc student in the University of Montreal Centre for the Study of Animal Reproduction, "*Proliferation Dynamics of Carnivore Embryos During Entry and Escape From Obligate Diapause*"

P-18. Naif Alhathal, PhD student in the McGill Department of Urology, "*Is Sperm DNA Damage Associated with Poor IVF Embryo Quality? A Systematic Review*" and "*Effect of Microsurgical Varicocelectomy on Human Sperm DNA Integrity and Chromatin Compaction*"

11:45:12:15 **Dr. Martine Culty, Department of Medicine Research Institute, McGill University Health Centre**, "*The missing chapter: what happens before the spermatogonia?*" – introduced by Makoto Nagano

12:15-1:00 *Lunch (provided)*

1:00-1:45 **Dr. Catherine Klein, President-Elect 2011 Environmental Mutagen Society, Department of Environmental Medicine, NYU School of Medicine**, "*Gene silencing by Estrogens and other Xenobiotics*" – introduced by Barbara Hales

1:45-3:00 Oral presentations (Chairs: **Geraldine Delbes and Aimee Ryan**)
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O-08. **Isabelle Demeestere, Department of Obstetrics and Gynecology**, **McGill University.** "*Role of follicle-stimulating hormone in the acquisition of oocyte developmental competence*"

3:00-3:30 *Health break*

3:30-4:15 **Dr. Daniel Carson, Schlumberger Chair of Advanced Studies and Research, Professor of Biochemistry and Cell Biology, Rice University**, "*MUC1: Functions and Opportunities in Reproductive Medicine*"– introduced by Daniel Dufort

4:15 -4:45 **Dr. Daniel Bernard, Department of Pharmacology and Therapeutics, McGill University**, "*Mechanisms of FSH synthesis: The story of a pig and a FOX*" – introduced by Cynthia Goodyer

4:45-5:00 Award Presentation (best oral presentation, best poster) and Concluding Remarks: **Hugh Clarke**

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

HUMAN REPRODUCTION AND DEVELOPMENT

NOUVELLES AVANCÉES
EN REPRODUCTION ET DÉVELOPPEMENT

Journée de recherche 2011

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

le mercredi 4 mai 2011
McGill New Residence Hall, 3625 avenue du Parc, Montréal, Québec

Pour s'inscrire
Veuillez remplir le formulaire d'inscription et le faire parvenir à csr-cer@mcgill.ca
or par télécopieur à Aliea Jamal au (514) 398-7120

Date limite de soumission des résumés : le 1 avril 2011

Date limite d'inscription : le 20 avril 2011

8 h — Inscription et café / Installation des affiches

8 h 45 — Mot de bienvenue : **Dr. Bernard Robaire**

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12 h 15 *Dîner (fourni)*

13 h **Dr. Catherine Klein, President-elect 2011 Environmental Mutagen Society, Department of Environmental Medicine, NYU School of Medicine**, "*Gene silencing by Estrogens and other Xenobiotics*" – présentée par Barbara Hales

13 h 45 Présentations orales (Modérateurs : **Geraldine Delbes et Aimee Ryan**)
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15 h *Pause café*

15 h 30 **Dr. Daniel Carson, Schlumberger Chair of Advanced Studies and Research, Professor of Biochemistry and Cell Biology, Rice University**, "*MUC1: Functions and Opportunities in Reproductive Medicine*"– présentée par Daniel Dufort

16 h 15 **Dr. Daniel Bernard, Department of Pharmacology and Therapeutics, McGill University**, "*Mechanisms of FSH synthesis: The story of a pig and a FOX*" – présentée par Cynthia Goodyer

16 h 45 Présentation de prix (meilleure affiche, meilleure présentation orale) et mot de conclusion : **Hugh Clarke**

17 h Démontage des affiches

Invited Speakers
Présentateur et présentatrices invités

Dr. Haifan Lin, Professor of Cell Biology and Genetics**,** Director of Yale University Stem Cell Center (9:00 – 9:45 a.m.) "*A Novel Small RNA-Mediated Epigenetic Mechanism Related to Stem Cells*"

Dr. Martine Culty, Department of Medicine Research Institute, McGill University Health Centre (11:45 – 12:15 p.m.) "*The missing chapter: what happens before the spermatogonia?*"

Dr. Catherine Klein, President-Elect 2011 Environmental Mutagen Society, Department of Environmental Medicine, NYU School of Medicine (1:00 – 1:45 p.m.) "*Gene silencing by Estrogens and other Xenobiotics*"

Dr. Daniel Carson, Schlumberger Chair of Advanced Studies and Research, Professor of Biochemistry and Cell Biology, Rice University (3:30 – 4:15 p.m.) "*MUC1: Functions and Opportunities in Reproductive Medicine*"

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A Novel Small RNA-Mediated Epigenetic Mechanism Related to Stem Cells

Haifan Lin

Yale Stem Cell Center, Yale University, USA

Small non-coding RNAs have emerged as key players in epigenetic regulation. We and others independently discovered in 2006 a novel class of small RNAs that interact with Piwi proteins in the mammalian and *Drosophila* germline. These Piwi-interacting RNAs (piRNAs), mostly 26-32 nucleotide in length and correspond to all types of genomic sequences, represent a distinct small-RNA pathway that is widely thought to function only in the germline. In this talk, I will review our recent work on the epigenetic function of the *Drosophila* Piwi protein and its associated piRNAs in somatic cells. This work reveals a novel epigenetic mechanism mediated by Piwi and its associated piRNAs in somatic cells that might also be applicable to the germline. Based on these results, we propose a "Piwi-piRNA guidance hypothesis" for Piwi proteins and piRNAs in epigenetic programming, i.e., the Piwi-piRNA complex serves as a sequence-recognition machinery that recruits epigenetic effectors such as Heterochromatin Protein 1a (HP1a) to specific sites in the genome to execute epigenetic regulation. Furthermore, our study demonstrates that the PIWI-piRNA pathway is related to stem cell self-renewal.

The missing chapter: what happens before the spermatogonia?"

M. Culty

Department of Medicine, Montreal General Hospital, McGill University, Canada

Male germ cell development comprises two main phases, a fetal/neonatal phase leading to the formation of spermatogonial stem cells (SSCs), and the spermatogenic cycle, a tightly regulated succession of cell division and differentiation leading to the production of spermatozoa. The germ cell lineage emerges in the embryo with a few epiblastic cells becoming primordial germ cells and migrating to the genital ridge, where somatic cells surround them during seminiferous cord formation. It is at this point that germ cells are identified as gonocytes, a term coined in 1957 by Clermont & Perey to described fetal germ cells resident in the gonad primordia and further extended, based on morphological criteria, to include neonatal germ cells. The terms pre- and pro- spermatogonia appeared later on, identifying germ cells at the period preceding spermatogonia formation. This time period, encompassing germ cells at different phases of development that are morphologically undistinguishable, has been scarcely studied compared to later phases of germ cell development. Understanding the mechanisms regulating gonocyte development is critical since the disruption of this process can jeopardize SSC formation and lead to infertility or tumor formation.

In rat, following several days of quiescence, gonocytes become mitotically active at postnatal day (PND) 3 and 4, and migrate to the basement membrane of the seminiferous cords where they differentiate into spermatogonia. Our laboratory studies the mechanisms regulating gonocyte proliferation and differentiation, the effects of endocrine disruptors on these processes and how their disruption might lead to infertility or tumor formation. We have shown that PDGF and 17β-estradiol (E2), act in concert, via ERK1/2 activation, to stimulate *in vitro* neonatal rat gonocyte proliferation, and that exogenous estrogenic compounds have effects similar to E2. I*n utero* exposure to exogenous estrogens increased PDGF receptors (PDGFRs) and MAPK expression in neonatal testis and transiently increased spermatogonia numbers at prepuberty. We further found that rat gonocyte differentiation was induced by retinoic acid (RA), in a manner dependent of PDGFR activation. Concomitant to the mRNA increase of the differentiating spermatogonia marker Stra8, RA increased the expression of variant forms of PDGFRs. Several germ cell and stem cell markers were differentially affected by PDGF/E2 treatment, providing clues on the molecular changes taking place during the transition from proliferating to differentiating gonocytes. In an effort to characterize neonatal gonocytes, we performed a comparative gene expression analysis of PND3 gonocytes and PND8 spermatogonia. Genes highly expressed in gonocytes were identified, which might correspond to genes down-regulating during differentiation. Some of these genes were also highly expressed in testicular germ cell tumors, representing good candidates to follow in search of the origins of germ cell tumors. These studies have been instrumental in understanding gonocyte function and broadening our knowledge of this seldom studied phase of male germ cell development.

Gene silencing by Estrogens and other Xenobiotics

Catherine B. Klein, PhD.

NYU Langone Medical Center, New York, NY

Epigenome modulation occurs over a lifetime, with known influences by a growing list of environmental exposures, diet and lifestyle exposures. My lab has developed and exploited a novel stable transgene reporter assay that allowed the concurrent study of a mixture of mutagenic and epigenetic gene silencing processes. Using the *gpt* transgenic G12 cells, we were the first to demonstrate the predominant epigenetic mode of carcinogenic nickel compounds. Subsequently, my lab was the first to demonstrate an epigenetic gene silencing component for another carcinogenic metal, hexavalent chromium. Ongoing studies have shown the G12 cell system to be informative for other environmental and pharmaceutical exposures as well, including X-rays and diethylstilbestrol (DES), Further studies in the lab have focused on epigenetic outcomes of exposures to arsenic at doses that are not highly mutagenic. We demonstrate prolonged epigenetic modulation in G12 and human skin cells after a single brief exposure to arsenite, with continuing modulation of epigenetic effects on gene expression even in the absence of any further arsenic exposure, and with evidence of ongoing genome instability.

MUC1: Functions and Opportunities in Reproductive Medicine

Daniel D. Carson, Department of Biochemistry and Cell Biology, Rice University, Houston, TX 77005 and Department of Biochemistry and Molecular Biology, M. D. Anderson Cancer Center, Houston, TX 77030

The apical surface of uterine epithelia is dominated by high molecular weight mucin glycoproteins. These mucins play important constitutive roles in protecting mucosa from microbial and enzymatic attack as well as lubrication. Moreover, the generally antiadhesive nature of these large molecular weight molecules prevents adhesion of apposing cell surfaces. In the context of embryo implantation, the prototypical transmembrane mucin, MUC1, has been shown to be antiadhesive with regard to blastocyst attachment and is lost or drastically reduced from the apical cell surface at the time of embryo attachment in all species studied to date. Expression of the MUC1 gene is controlled in a complex fashion by the integrated and sometimes synergistic actions of progesterone receptor and cytokine-activated transcription factors. Recent studies have indicated that agonists of peroxisome proliferator-activated receptor-γ (PPARγ) inhibit MUC1 gene and protein expression in multiple contexts. In addition, the availability of a battery of MUC1 monoclonal antibodies and the high accessibility of MUC1 at the cell surface make it an excellent candidate for antibody-directed, nanoparticle based therapies. MUC1 is a member of a family of transmembrane mucins expressed by uterine epithelia in normal and pathological contexts. Consequently, developing methods to control mucin expression provides novel opportunities to enhance the normal protective functions of the uterine mucosa and to enhance embryo implantation success. In addition, mucin glycoproteins provide new therapeutic targets in endometrial pathologies including cancer and endometriosis. (supported by NIH grants R01HD29963 [DDC] and P50CA098258 [DDC].

Mechanisms of FSH synthesis: The story of a pig and a FOX

Daniel Bernard

Department of Pharmacology and Therapeutics, McIntyre Medical Building, McGill University, Canada

Follicle-stimulating hormone (FSH) is a critical regulator of gonadal function, particularly in females. Activins stimulate FSH synthesis and secretion by increasing transcription of the FSH β subunit gene (*Fshb*) in pituitary gonadotrope cells. Comparative analyses of the *Fshb/FSHB* promoters in mouse, pig, and humans led to the identification of the forkhead transcription factor, FOXL2, as a critical mediator of the activin response. In this lecture, we will discuss: 1) the *in vitro* data that led to a model for FOXL2 regulation of *Fshb* transcription, and 2) recent analyses of fertility and FSH synthesis in gonadotrope-specific *Foxl2* knockout mice.

Oral Presentations
Présentations orales

1. Nathalie Bedard (9:45 a.m.)
2. Ava Schlisser (10:00 a.m.)
3. Genevieve Plante (10:15 a.m.)

4. Lisanne Grenier (1:45 p.m.)
5. Malena Rone (2:00 p.m.)
6. Michelle Collins (2:15 p.m.)
7. Macalister Usongo (2:30 p.m.)
8. Isabelle De Meestere (2:45 p.m.)

Mice Lacking the USP2 Deubiquitinating Enzyme have Severe Male Sub-Fertility Associated with Defects in Fertilization and Sperm Motility

Nathalie Bédard[1], Yaoming Yang[1], Mary Gregory[5], Daniel G. Cyr[5], João Suzuki[2], Xiaomin Yu[2], Ri-Cheng Chian[2], Louis Hermo[3], Cristian O'Flaherty[4], Charles E. Smith[3,6], Hugh J. Clarke[2], and Simon S. Wing[1*]

The ubiquitin-proteasome system plays an important role in spermatogenesis. However, the functions of deubiquitinating enzymes in this process remain poorly characterized. We previously showed that the deubiquitinating enzyme USP2 is induced in late elongating spermatids. To identify its function, we generated mice lacking USP2. *Usp2 -/-* mice appeared normal and the weights of major organs including the testis did not differ from wild type (*Usp2 +/+)*. However, although the numbers of testicular spermatids and epididymal spermatozoa were normal in *Usp2 -/-* males, these animals had a severe defect in fertility, yielding only 12% as many offspring as *Usp2 +/+* littermates. Spermatogenesis in *Usp2 -/-* mice was morphologically normal except for the presence of abnormal aggregations of elongating spermatids and formation of multinucleated cells in some tubules. The epididymal epithelium was morphologically normal in *Usp2 -/-* mice, but some abnormal cells other than sperm were present in the lumen. *Usp2 -/-* epididymal spermatozoa manifested normal motility when incubated in culture media, but rapidly became immotile when incubated in phosphate buffered saline in contrast to *Usp2 +/+* spermatozoa which largely maintained motility under this condition. *Usp2 -/-* and *+/+* spermatozoa underwent acrosome reactions *in vitro* with similar frequency. *In vitro* fertilization assays demonstrated a severe defect in the ability of *Usp2 -/-* spermatozoa to fertilize eggs. This could be bypassed by intracytoplasmic sperm injection or removal of the zona pellucida which resulted in fertilization rates similar to that of *Usp2 +/+* mice. We demonstrate for the first time using mouse transgenic approaches, a role for the ubiquitin system in fertilization.

HYDROXYUREA-INDUCED CAUDAL MALFORMATIONS IN MICE ARE ENHANCED BY DEPRENYL

Ava Schlisser[1] and Barbara Hales[1]
[1]Department of Pharmacology and Therapeutics, McGill University, Montreal, Quebec, Canada

Hydroxyurea (HU) is a developmental toxicant in mammalian embryos; treatment of mice on gestation day 9 (GD 9) with HU produces defects of the caudal region, including hindlimb, vertebral, and tail malformations. These malformations are associated with oxidative stress during organogenesis. HU increases the formation of reactive oxygen species (ROS) and the production of 4-hydroxy-2-nonenal (4-HNE), a cytotoxic product of lipid peroxidation. 4-HNE forms protein adducts that are concentrated in the caudal region. Proteomic data reveal that 4-HNE targets glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in the embryo. HU treatment decreases the catalytic activity of GAPDH and leads to its translocation from the cytoplasm into the nucleus. We hypothesize that inhibition of the nuclear translocation of GAPDH will attenuate HU-induced caudal malformations. Deprenyl (Selegiline®), a drug that is used to treat Parkinson's disease because of its action as an irreversible and selective monoamine oxidase B (MAO-B) inhibitor, inhibits the nuclear translocation of GAPDH. To test our hypothesis, CD1 mice were treated with vehicle (saline) or deprenyl (Sigma-Aldrich Co.) at 10 mg/kg by i.p. injection at 8:00 AM on GD 9. After 1 h, female mice were treated with saline or HU (400 or 600 mg/kg) by i.p. injection. Dams were euthanized on GD 18 and fetuses were inspected for external and skeletal malformations. Exposure to either saline or deprenyl alone did not affect progeny outcome. Treatment with HU alone at 400 or 600 mg/kg resulted in hindlimb malformations (12.5% or 28.0%, respectively) and curly/hypoplastic tails (2.1% or 60%, respectively). Exposure to HU and deprenyl increased the incidence of caudal malformations; hindlimb malformations increased to 34.4% (HU 400 mg/kg plus deprenyl) or 58.8% (HU 600 mg/kg plus deprenyl), whereas curly/hypoplastic tails increased to 34.4% or 76.4%, respectively, in the presence of deprenyl. Since the inhibition or absence of MAO-A/B during mouse embryo development is not associated with developmental abnormalities, it is likely that the effects of deprenyl are due to the inhibition of GAPDH nuclear translocation. We suggest that 4-HNE-GAPDH serves to protect the embryo from HU-induced caudal malformations and may act as a stress sensor to the cell. Supported by CIHR.

BINDING AND FUNCTIONAL CHARACTERISTICS OF RECOMBINANT MURINE BINDER OF SPERM (BSP) PROTEINS.

Geneviève Plante BSc, Isabelle Thérien PhD, Puttaswamy Manjunath PhD
HMR Research Center, University of Montréal, Montréal, Québec, Canada

Sperm capacitation is a maturation step that is essential in order for sperm to be able to fertilize an oocyte. A family of proteins, the Binder of Sperm (BSP), are known to bind choline phospholipids in sperm membrane and thus promote capacitation in many species. These proteins, secreted by the seminal vesicles, share similar characteristics such as binding to gelatine, heparin and glycosaminoglycan (GAG). Recently, BSP−homologous genes have been identified in the epididymis of human (BSPH1) and mouse (Bsph1 and Bsph2a). The objective of the current study was to determine if Bsph1 share some binding and functional characteristics with the other BSP proteins and therefore could play a similar role in sperm functions. Because a very small amount of BSP homologs are found in human and mouse, we produced recombinant Bsph1 (rBsph1). The rBsph1 was then tested for binding to GAG, gelatine, heparin, liposomes of phosphatidylcholine and sperm membranes. Finally, rBsph1 was tested for its effect on mouse sperm capacitation. The preliminary data indicate that rBsph1 binds to gelatine, heparin, phosphatidylcholine and sperm membranes on the acrosome region and the midpiece. The results also indicate that the murine rBsph1 promotes capacitation. These results indicate that the BSP proteins secreted by the epididymis could have similar role in sperm function as the one secreted by seminal vesicles. (Supported by NSERC, CIHR and FESP of university of Montréal)

Paternal exposure to cyclophosphamide leads to the formation of micronuclei and a dampened DNA repair response in 8 cell embryos

Lisanne Grenier, Bernard Robaire, Barbara F. Hales.

The treatment of male rats with cyclophosphamide (CPA), a commonly used anticancer alkylating agent, results in adverse progeny outcomes that range from increased pre- and post-implantation loss to growth retardation and congenital malformations. The DNA damage introduced by CPA in male germ cells is recognized by maternally stored repair proteins in the early zygote. Phosphorylated H2AX (γH2AX) foci, a mark of DNA double strand breaks, are increased biphasically in the male pronuclei of zygotes sired by CPA treated males. Poly(ADP-ribose) polymerase-1(PARP-1), activated by both single and double strand DNA breaks, is substantially elevated in both parental genomes in the zygote. We hypothesize that the ability of subsequent cleavage stages embryos to activate a repair response will determine their fate. We used two markers to assess DNA repair capacity. The first, 53BP1, is a component of the non-homologous end joining repair pathway. The second, poly(ADP-ribose) polymer (PAR), is the product of PARP activity and recruits the DNA repair proteins involved in homologous recombination and base excision repair pathways to sites of damage. The formation of micronuclei was used as a measure of DNA damage accumulation and outcome of the repair process. Male Sprague-Dawley rats were gavaged daily with saline or 6mg/kg/day of CPA for 4 weeks prior to mating to two naturally cycling pro-estrous females. Eight cell embryos were collected on gestation day 3 and prepared for the detection of 53BP1 and PAR by immunofluorescence; DNA was counterstained with propidium iodide. Images were acquired using confocal microscopy and analyzed with Imaris software. 53BP1 immunoreactivity was found homogenously distributed in nuclei and in the endoplasmic reticulum. The mean intensity of nuclear 53BP1 was lower by almost 50% in the embryos sired by CPA treated males compared to control 8 cell embryos. PAR was also homogenously distributed in nuclei and in the endoplasmic reticulum. Embryos sired by CPA-treated males displayed a decrease in PAR nuclear mean intensity, attaining only 50% of the control. This apparent decrease in DNA repair response was accompanied by a dramatic increase in DNA damage or genetic instability. While micronuclei were found in only 4% of control 8 cell embryos, the incidence of micronuclei in embryos sired by CPA exposed males was elevated to 83%. This inability of the embryo to adequately repair the paternal genome is likely to contribute to the elevated pre- and post-implantation death observed as a consequence of paternal CPA exposure. Decreases in 53BP1 immunoreactivity and PARP activity may reflect a decrease in the transcription of DNA repair proteins in the early embryo. Supported by CIHR.

Mechanism of MITOCHONDRIAL Cholesterol Transport by the Steroidogenic Acute Regulatory Protein

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Cholesterol transfer from the outer mitochondrial membrane (OMM) to the inner mitochondrial membrane (IMM) is the rate-limiting and hormone-sensitive step in the acute regulation of steroid biosynthesis. This lipid transfer is accomplished by the formation of a multimeric protein complex anchored at the OMM by the translocator protein (TSPO), interacting with voltage-dependent anion channel (VDAC) and IMM protein, Adenine Nucleotide Translocase (ANT), which are proposed to form an OMM/IMM contact site. Hormone-induced synthesis of the Steroidogenic Acute Regulatory protein (STAR) has been proposed to initiate the transfer of cholesterol from OMM to IMM enzyme CYP11A1, which cleaves cholesterol to pregnenolone. To evaluate the regulation and formation of this protein complex in control and hCG stimulated MA-10 mouse Leydig cell mitochondria we utilized Blue Native Polyacrylamide Gel Electrophoresis (BN-PAGE) followed by mass spectrometry (MS). Through BN-PAGE analysis, TSPO was identified to be present in 66-kDa and 800-kDa protein complexes as a monomer and polymer respectively, with an increase in the 800-kDa complex after hCG treatment. Through MS analysis the 800-kDa complex was shown to contain VDAC, ANT and CYP11A1; whose expression increased upon hCG stimulation, suggesting that this complex forms a functional IMM/OMM contact site for cholesterol transfer. This was confirmed through the use of [^{3}H]photocholesterol, which was incubated with mitochondria isolated from control and hCG-treated cells prior to solubilization for BN-PAGE and crosslinked by UV-irradiation. The radiolabeled cholesterol bound to both the 66- and 800-kDa complexes. Addition of recombinant full length, but not C-27, STAR to isolated mitochondria resulted in time-dependent changes in the amount of crosslinked cholesterol at the 800-kDa complex. These results suggest that hormone-induced polymerized TSPO present in mitochondrial contact site binds cholesterol that is able to be mobilized by STAR to the IMM for processing by CYP11A1. (Supported by CIHR MOP-102647 and NIH ES 007747).

ASYMMETRIC EXPRESSION OF CLAUDIN-10 DIRECTS PATTERNING OF THE LEFT-RIGHT AXIS

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In vertebrates, the asymmetric positioning of internal organs relative to the midline is evolutionarily conserved, and required for normal physiological function. The genetic cascade that defines asymmetric organ positioning initiates during gastrulation, during which several ions including calcium, have been implicated in the initial symmetry breaking event. The mechanisms by which these events are translated into asymmetric gene expression remain unknown. We previously examined the role of the integral tight junction protein, *Claudin-1*, in patterning the left-right axis in the chick, and showed that overexpression of Claudin-1 on the right side of the embryo randomized the direction of heart looping. To identify other claudins expressed during gastrulation, we performed expression analyses of the claudin family. We identified that 15 claudins exhibit unique and/or overlapping expression patterns during gastrulation. Interestingly, we discovered that *Claudin-10* was asymmetrically expressed at Hensen's node, the site where asymmetric gene expression is first observed in the chick embryo and where the initial symmetry breaking events required for left-right patterning occur. Overexpression of *Claudin-10* on the left side of the node, or knockdown of endogenous *Claudin-10* on the right side of the node, caused a significant increase in abnormal leftward heart looping, indicative of abnormal left-right patterning. We also observed alterations in the expression of the classic left-right morphogenesis gene, *Pitx2c*, in these embryos. We are currently examining the effects of manipulating *Claudin-10* expression on other classic left-right patterning genes, and identifying the functional domains necessary for the role that *Claudin-10* plays during left-right patterning. These data suggest that asymmetric expression of *Claudin-10* is required for normal left-right patterning, perhaps through regulation of the permeability of the ions that have been proposed to be involved in the initial symmetry-breaking event that occurs at Hensen's node.

Beta-catenin/Tcf expression of lacZ-transgene in OSE cells is inhibited downstream of nuclear localization of beta-catenin in TopGal mice

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In response to activation of the Wnt signaling pathway, active beta-catenin accumulates in the nucleus, where it associates with LEF/TCF (lymphoid enhancer factor/T-cell factor) transcription factors to activate gene expression. Using a β-catenin/Tcf-responsive reporter mouse (TopGal), we had identified a β-galactosidase positive (lacZ+) ovarian surface epithelial (OSE) cell population that overlies the medio-lateral surface of mouse indifferent gonad and demonstrated an age-dependent decrease to relative constancy in proportion of stained to unstained cells when animal reached maturity. We investigated whether age-dependent decrease in lacZ+ OSE cells is due to differential expression of Wnt signaling-related components within OSE. RT-PCR for Wnt signaling components indicated Wnts/frizzleds are expressed throughout OSE development. Immunohistochemical staining for active beta-catenin localized dephosphorylated beta-catenin on plasma membrane of OSE cells. Lithium chloride, a Wnt signal activator by GSK-3β inhibition, stabilized free beta-catenin and lead to nuclear accumulation of active beta-catenin in OSE cells *in vitro*. Unexpectedly, we could not detect reporter activity in non-signaling cells following stimulation. Finally, Chibby, an inhibitor of beta-catenin/Tcf expression, was localized in OSE cells and could be a potential inhibitor of beta-catenin/Tcf expression in OSE cells. Together, this study shows that active beta-catenin accumulation and nuclear localization is not indicative of transcriptional activity in OSE cells.

Role of follicle-stimulating hormone in the acquisition of oocyte developmental competence

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Follicle-stimulating hormone (FSH) plays a crucial role during follicular development by supporting antral follicular growth until preovulatory stage. Ovaries from mice lacking the β-subunit of FSH do not display follicles beyond the early antral stage, leading to anovulation and sterility. However, we have found that oocytes from *Fshb*$^{-/-}$ mice reached same size as those of wild-type, indicating that the oocyte growth in vivo is FSH-independent. Next, we investigated the role of FSH in the acquisition of oocyte developmental competence. Growing oocytes progressively acquire the ability to undergo meiotic maturation. In oocytes of *Fshb*$^{-/-}$ mice, this ability was acquired relatively slowly. but ultimately matched that of wild-type oocytes. Following in vitro fertilization, eggs from *Fshb*$^{-/-}$ mice developed poorly compared to wild-type, even when FSH and hCG were supplied to induce late antral folliculogenesis and ovulation *in vivo*. Fertilization by intracytoplasmic injection significantly improved the development of eggs from *Fshb*$^{-/-}$ mice, although it remained lower than wild-type. These results show that oocyte growth and acquisition of the ability to undergo maturation do not require FSH. However, FSH seems to play an as yet undefined role in acquiring or maintaining developmental competence.

Poster Presentations
Présentations par affiches

1. France-Helene Paradis
2. Gupreet Manku
3. Tania Morielli
4. Marie-France Lusignan
5. Halim Khairallah
6. Oli Sarkar
7. Andrew Midzak
8. Ahmed Barry
9. Dora Siontas
10. Craig Park
11. Sathvika Sanjiv Jagannathan
12. Qin Yang
13. Amanda Baumholtz
14. Christopher Garcia
15. Dyananda Siddappa
16. Tina Djogo
17. Evelyn Llerena
18. Naif Alhathal

EFFECTS OF VALPROIC ACID AND VALPROMIDE ON LIMB MORPHOLOGY AND GENE EXPRESSION DURING DEVELOPMENT CORRELATE WITH THEIR HISTONE DEACETYLASE INHIBITION ACTIVITY

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In utero exposure to valproic acid (VPA), a histone deacetylase (HDAC) inhibitor, induces neural tube and skeletal malformations. Valpromide (VPD), an analog of VPA, is less potent as an HDAC inhibitor as well as less teratogenic than VPA. Therefore, we hypothesized that the HDAC inhibitor activity of VPA is responsible for its teratogenicity. In this study, we use an *in vitro* limb bud culture system to compare the effects of increasing concentrations of VPA and VPD on limb differentiation and morphology, histone acetylation and the expression of two genes important during development, Sox9 and Hypoxia-inducible factor (Hif1a). VPA significantly inhibited limb growth and differentiation, increased histone 4 acetylation and downregulated Sox9 and HIF1a transcripts at 3h in all treatment groups. In contrast, VPD caused a significant reduction in growth only in the highest concentration group and did not show any significant effect on neither acetylation of histone 4 nor expression of Sox9 and Hif1a. Together, these results suggest that the teratogenicity of VPA is correlated with its activity as an HDAC inhibitor and is associated with the disregulation of Sox9 and Hif1a during limb development. These studies were supported by CIHR and FRSQ.

From gonocyte differentiation to testicular tumors: where is the link?

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Testicular cancer, in particular testicular germ cell tumor (TGCT), is the most common form of cancer in young men. However, very little is known about the underlying causes of TGCT. It is believed that TGCTs arise from primordial germ cells or gonocytes that failed to adequately differentiate. We study gonocyte development and have found that PDGF is involved in gonocyte proliferation (1). We also identified a truncated form of PDGFR in gonocytes that is increased upon retinoic acid treatment (2). Interestingly, TGCTs were found to express variant forms of PDGFRs. One of our goals is to determine whether disruption of PDGFR expression or function could play a role in TGCT formation or whether the presence of variant PDGFRs in TGCTs simply reflects the retention of a fetal phenotype by the tumor cells. We also want to identify other genes involved in gonocyte development that might be related to germ cell tumor formation. To this end, we performed a comparative analysis of the gene expression profiles of gonocytes and spermatogonia as well as in a panel of human normal and TGCTs, including the human TCam-2 seminoma cell line. We found that TCam-2 cells and TGCT biopsies, but not normal testicular tissue, express a 1.5kb PDGFRα variant previously identified in TGCTs. Northern blot analysis of neonatal rat testis showed that it also contains a 1.5kb PDGFRα transcript. A number of similarities between gonocytes and Tcam-2 cells were found at the protein and RNA levels. Gene array comparisons identified several genes that were highly expressed in gonocytes as compared to spermatogonia and were overexpressed in TGCTs, suggesting that these genes failed to be down-regulated in tumor germ cells as they were during normal differentiation. The similarities between gene and/or protein expression profiles between gonocytes and TGCTs support the idea that TGCTs may arise from a disruption of early male germ cell development and might provide new gene targets to further study in relation to TGCT formation.

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PROTEIN TYROSINE NITRATION IS MODULATED BY REACTIVE OXYGEN SPECIES AND DIFFERENTIALLY LOCALIZED IN HUMAN SPERMATOZOA

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Oxidative stress generated by excessive reactive oxygen species (ROS) and/or decrease in antioxidant defences, is associated with male infertility. A consequence of oxidative stress is the production of tyrosine nitration (NO_2-Tyr), a protein modification which is generated by peroxynitrite ($ONOO^-$; product of $O_2^{\bullet-}$+NO•). We hypothesize that excessive NO_2-Tyr ~~may~~ have the effect of reducing sperm motility. The aims of this study were 1) to compare the effect of different ROS and antioxidants on NO_2-Tyr levels, 2) to determine the subcellular localization of NO_2-Tyr in human spermatozoa and 3) to determine the pattern of NO_2-Tyr in infertile men with asthenozoospermia. Percoll washed sperm samples from healthy donors were incubated for 30 minutes at 37^{o}C with 2mM H_2O_2 or tert−butyl hydroperoxide (tert−BHP), or 250µM DaNONOate (a NO• donor) in the presence or absence of 0.5mg/ml superoxide dismutase (SOD) or catalase (CAT), or 1mM reduced glutathione (GSH), and then the sperm proteins were immunobloted for NO_2-Tyr. The level of NO_2-Tyr in 78, 87, 107 and 182 kDa proteins was significantly higher in DaNONOate− treated spermatozoa when compared to controls. H_2O_2 or tert−BHP produced control level NO_2-Tyr. SOD and GSH decreased the level of NO_2-Tyr induced by DANONOate, while CAT caused levels to increase. Following fractionation, NO_2-Tyr proteins were found to be differentially localized in the cytosolic, Triton−soluble and –insoluble (TI) sperm fractions. The level of NO_2-Tyr was increased in all treated fractions when compared to controls. The TI fraction showed the highest increase, and was the only fraction containing the 107 kDa protein band. Spermatozoa from healthy donors with low motility (recovered from the 40-65% interface) contained higher levels of NO_2-Tyr than those with high motility (recovered from the 95% Percoll layer). NO_2-Tyr levels were also higher in spermatozoa from asthenozoospermic patients than in healthy donors, suggesting that increased NO_2-Tyr may be responsible for the sperm motility failure observed in male infertility. Funded by CIHR.

Studies of the interaction between Binder of SPerm1 (BSP1) and egg yolk LDL

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Introduction

Egg yolk is used in extender to protect sperm from cold shock and freezing during preservation. It is the low-density lipoprotein (LDL) fraction of egg yolk that protects sperm. The major proteins from bull seminal plasma, the Binder of SPerm (BSP) proteins, are detrimental to sperm preservation because they induce a continual phospholipids and cholesterol efflux from sperm membranes. The BSP proteins were proposed to bind to egg yolk LDL and this interaction is believed to shield sperm against the detrimental effect of BSP proteins. In the current investigation, this putative association was characterized by isothermal titration calorimetry.

Methods

BSP1 was purified from bull seminal plasma by affinity chromatography. LDL was isolated from egg yolk by ultracentrifugation using a KBr solution. We characterized the binding between BSP1, the major protein from the BSP protein family, and LDL by isothermal titration calorimetry.

Results

The association between BSP1 and LDL was characterized by an affinity constant (K_a) of $3.4 \pm 0.4\ \mu M^{-1}$. It was estimated that 104 molecules of BSP1 could bind one LDL particle. This stoichiometry leads to proposing that the association involves 1.6 ± 0.1 phosphatidylcholines (PC) per BSP protein, suggesting that the PC molecules of the LDL monolayer act as binding anchors for the BSP1 proteins and that proteins from the LDL surface may also be a part of a binding site.

Conclusion

In conclusion, the formation of a high affinity complex between BSP1 and LDL seems to be the key to the sperm protection by egg yolk extender.

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The Role of Claudins in Kidney Morphogenesis

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Congenital kidney malformations are the most common cause of childhood renal failure. To understand how these defects arise, it is essential to identify and characterize molecules that are required for kidney formation. The Claudin family of integral tight junction proteins has documented roles in tissue and organ morphogenesis, including tubule and lumen formation and maintenance of epithelial cell layers. In the adult kidney, the overlapping expression patterns of individual Claudins correlate with differential paracellular transport properties along the nephron. However, the function of Claudin family members during kidney organogenesis has not been studied. Mutations in human Claudin-16 and Claudin-19 are associated with an autosomal recessive syndrome known as familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC) in which renal paracellular reabsorption of magnesium and calcium is impaired. Affected individuals develop end stage renal failure, but the reasons for this are unclear. Claudin-7 is also important for renal paracellular transport: Claudin-7 knockout mice die from salt wasting and dehydration in the first few weeks of life. Previous work from our laboratory has shown that Claudin-7, -16 and -19 are expressed during key stages of kidney development. We hypothesize that these Claudins are critical for normal kidney development and that kidney malformations will result when their expression is perturbed. To address this hypothesis, we will first perform an in-depth analysis of Claudin-7, -16 and -19 expression during kidney morphogenesis using RT-PCR, whole mount *in situ* hybridization, and immunohistochemistry. We will then assess their function by manipulating the expression of each Claudin in an *in vitro* cell culture model of tubulogenesis, in a chick *in ovo* model of kidney development, and in mouse embryonic kidney explants. The results will establish the roles of these Claudins during kidney development.

IS V1-PDGFRβ INVOLVED IN THE PROGRESSION FROM PROLIFERATION TO DIFFERENTIATION IN TESTICULAR NEONATAL GERM CELL?

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Spermatogenesis depends on the formation of spermatogonial stem cells (SSCs) from their precursor cells, the gonocytes. Rat neonatal gonocyte proliferation and migration occur between postnatal days (PND) 3 and 4 in rat and are followed by differentiation to spermatogonia. We have shown that platelet-derived growth factor BB (PDGF) and 17β-estradiol (E) stimulate gonocyte proliferation in a coordinated manner via Erk1/2 activation. We identified a gonocyte-specific variant of PDGF receptor β, V1-PDGFRβ whose expression increased during retinoic acid-induced differentiation, suggesting a role of V1-PDGFRβ in differentiation. The objectives of the present study were to determine the gene expression changes occurring during proliferation and to examine whether V1-PDGFRβ plays a role in the transition to differentiation. Because GDNF and FGF2 were previously reported to support gonocyte survival, we compared their effects to those of PDGF and E. To this end, PND3 gonocytes were cultured for 1 day in serum-free medium alone or with either PDGF + E or GDNF + FGF2. The expression of genes differentially expressed in gonocytes, SSCs and differentiating spermatogonia, as well as the status of V1-PDGFRβ were determined by quantitative real time PCR. While the expression of the suppressor of meiosis Nanos 2 and V1-PDGFRβ were decreased by PDGF + E, they were both increased by GDNF + FGF2. By contrast, both conditions induced similar increases in the RNA binding protein Dazl and decreases in the embryonic stem cell marker Nanog. In separate experiments, gonocytes were transfected with expression vectors encoding either for GFP or for a GFP-mV1-PDGFRβ fusion protein and the effects of mV1-PDGFRβ overexpression on gene expression were determined. While V1-PDGFRβ overexpression increased the mRNA level of Stra8, a marker of differentiating spermatogonia, the addition of PDGF + E reduced Stra8 expression independently of V1-PDGFRβ levels in the cells. These results suggest that PDGF and E not only stimulate proliferation but that they might also play a role in the maintenance of gonocyte phenotype, including a low level of V1-PDGFRβ expression. On the other hand, increased V1-PDGFRβ expression might be one of the factors regulating gonocyte differentiation and survival, in agreement with our previous findings.

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STRUCTURE-FUNCTION ANALSYS OF 5-ANDROSTEN-3β,17,19-TRIOL, A NOVEL LIGAND FOR THE MITOCHONDRIAL TRANSLOCATOR PROTEIN, AND RELATED STEROIDS WHICH CONTROL STEROIDOGENESIS

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Luteinizing hormone, through cAMP-mediated second messenger signaling, promotes testosterone synthesis in testicular Leydig cells by facilitating the transport of cholesterol to the mitochondrial matrix, where it is metabolized by the cytochrome P450 CYP11A1 to pregnenolone. The mitochondrial movement of cholesterol requires the action of the translocator protein (TSPO), which contains a C-terminal cholesterol recognition/interaction amino acid consensus (CRAC) domain that is essential for its function. We recently identified a novel TSPO ligand targeted to the CRAC domain, 5-androsten-3β,17,19-androstene-5-triol (19-Atriol), which was able to acutely control steroid biosynthesis by steroidogenic cells. To further our understanding of 19-Atriol and TSPO, we investigated the structure-activity relationship of a family of steroids with structural homology to 19-Atriol in the MA-10 mouse Leydig tumor cell model system. Structure-function analysis revealed that androstentriols hydroxylated at the 3, 16 and 17 positions of the sterol backbone had little effect on cAMP-mediated steroid synthesis. In contrast, potent inhibition of cAMP-mediated steroidogenesis was observed with androstenetriols hydroxylated at the 3, 17 and either the 4, 7 or 11 positions of the sterol backbone. Hydroxyl groups at the 3, 17 and 19 positions also appeared critical for the inhibitory action of 19-Atriol, as ketone substitutions at these positions markedly reduced the effectiveness of the 19-Atriol molecule at controlling cAMP-mediated steroidogenesis. Collectively, these findings suggest that the TSPO CRAC motif recognizes pharmacophores on the A and B rings of the sterol backbone and lays a foundation for the development of compounds that may be used to control diseases of aberrant steroid production. (Supported by CIHR MOP-102647 and TGF-36110).

EFFET DU bFGF SUR L'EXPRESSION ET LA PHOSPHORYLATION DE LA CONNEXINE
43 DES GAP JUNCTIONS DANS LES CELLULES FOLLICULOSTELLAIRES DE L'ANTÉ-
HYPOPHYSE ; ÉTUDE DES VOIES DE SIGNALISATION

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Les cellules folliculostellaires (FS) de l'anté-hypophyse (AH) modulent l'activité endocrine de la glande par leur sécrétion de cytokines et facteurs de croissance. Grâce à leurs prolongements cytoplasmiques et aux gap junctions constituées de connexine (Cx) 43, les cellules FS établissent des contacts entre-elles et avec les cellules endocrines pour contrôler l'activité sécrétrice de l'AH. Nous avons étudié ici les effets du facteur de croissance bFGF sur l'expression, la distribution et l'état de phosphorylation de la Cx43 en Ser368 (cible d'une PKC). Nous avons mesuré les taux de la Cx43 après traitement au bFGF dans les cellules TtT/GF, une lignée de cellules FS. Nos résultats montrent une cinétique bi-phasique ; le bFGF induit une augmentation suivi d'une chute du taux de la protéine. Le traitement à court terme au bFGF augmente la phosphorylation de la Cx43 en Ser368. Nos études en immunofluorescence révèlent une distribution périnucléaire de la Cx43 dans des conditions basales; après traitement au bFGF, la Cx43 augmente autour du noyau et dans les prolongements cytoplasmiques. Nous avons ensuite étudié les voies de signalisation activées par le bFGF susceptibles de participer à l'action du bFGF sur la Cx43. Nos résultats montrent une stimulation précoce de la voie Ras-Erk1/2-PLCγ-PKC. Notre étude indique l'implication d'une PKC conventionnelle dans la phosphorylation de la Cx43 en Ser368 induite par le bFGF. CRSNG

THE IDENTIFICATION OF PROTEINS THAT INTERACT WITH THE N-TERMINAL DOMAIN OF PITX2C

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During embryogenesis, a number of key events regulate the correct patterning of an organism, one of them being the correct positioning of internal organs. One of the factors important for this process is the homeodomain transcription factor Pitx2c, which is asymmetrically expressed in the lateral plate mesoderm. Since the N-terminal domain of Pitx2c is the only part that differs from the other isoforms, it is believed that it may play a role in left-right patterning. Previous work has shown that overexpression of the N-terminus randomizes the direction of heart tube looping, suggesting that this domain is critical for Pitx2c's function in left-right patterning. We hypothesize that the overexpressed N-terminus is antagonizing the activity of endogenous Pitx2c by competing for binding to critical interaction partner. In order to better understand the role of the N-terminal domain, I have performed yeast two-hybrid library screens to identify proteins that interact with this domain and I will characterize the expression of these proteins in mouse and chick embryonic tissue. Two different yeast two-hybrid library screens were performed. In the first, we used the chick Pitx2c N-terminal domain, which was cloned into the pGBKT7 vector in-frame with the DNA binding domain of the GAL4 transcription factor as bait, against a mouse adult cDNA library cloned in-frame with the GAL4 activation domain in the pGADT7 vector. In the second, we used a mouse Pitx2c N-terminal domain against an embryonic E11 mouse library. From the resulting interacting proteins, we are currently analyzing one candidate common to both screens and several candidates pulled from the screen using the embryonic mouse library. The interactions are being confirmed with a GST pull-down assay and their expression patterns are being characterized and compared to that of Pitx2c in mouse and chick embryos with *in situ* hybridization and immunohistochemistry.

UTERINE-SPECIFIC NODAL DELETION DISRUPTS PLACENTATION AND CONTRIBUTES TO PRETERM BIRTH IN MICE

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Pre-term birth (PTB) is the leading cause of perinatal mortality, accounting for over 75% of perinatal death. Despite recent progress, PTB has continued to rise over the years and remains an important clinical dilemma worldwide. Failure to decrease the rates of PTB is attributed, in part, to our lack of understanding of the causes and mechanisms that underlie pre-term delivery. In order to aid in the ongoing pursuit of elucidating these mechanisms, our laboratory has been characterizing the expression of the TGF-β superfamily member, Nodal, in the uterus and investigating the potential role this factor may play in facilitating the birth of healthy offspring.

Utilizing the loxP-Cre recombinase system, we have generated a conditional knockout of Nodal in the female reproductive tract of adult mice (Progesterone Receptor-Cre), bypassing embryonic lethality. Interestingly, the Nodal deficient mice exhibit various reproductive abnormalities, including reduced rates of establishing pregnancy, intrauterine growth restriction (IUGR) and preterm delivery late into development (d17.5). The placenta of the Nodal conditional knockout mothers exhibits significant disruption of the maternal basal plate by d12.5 and, coincidentally, resembles the morphology of a late-stage placenta. Furthermore, apoptosis is dysregulated at the maternal-fetal interface. We hypothesize that deleting uterine Nodal affects placentation and disrupts the endocrinological framework that underlies labour thereby inducing premature delivery in our mouse model.

We report here, a detailed phenotypic characterization of a uterine Nodal knockout strain, implicating the Nodal signalling pathway in facilitating healthy pregnancy. Our observations indicate that Nodal ligand from a maternal source may play a crucial role in decidualization and proper placenta development and its absence leads to IUGR and PTB. Understanding the mechanisms that underlie IUGR and pre-term delivery are paramount in the ultimate goal of eliminating complications during pregnancy leading to pre-eclampsia, PTB and embryonic loss.

VISUALIZATION OF THE DYNAMIC FORMATION OF A MITOCHONDRIAL CHOLESTEROL TRANSPORT COMPLEX BY FÖRSTER RESONANCE ENERGY TRANSFER CONFOCAL MICROSCOPY

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Abstract

The rate-limiting and hormone-sensitive step in steroid biosynthesis is the transfer of cholesterol from intracellular stores into the mitochondria. This step is mediated by a series of protein-protein interactions occurring at the outer mitochondrial membrane (OMM) leading to cholesterol import into the OMM and the subsequent transfer to the inner mitochondrial membrane (IMM) where the cytochrome P450 side chain cleavage enzyme of cholesterol (CYP11A1) is located which cleaves cholesterol to pregnenolone, the precursor of all steroids. Four mitochondrial membrane proteins have been identified to participate in this process, the translocator protein (18 kDa; TSPO) and the voltage-dependent anion channel (VDAC1) located in the OMM, the adenine nucleotide transporter (ANT1) and CYP11A1 found in the IMM. The close proximity of these proteins during hormone-induced steroidogenesis has been suggested *in vitro* biochemical protein-protein interaction studies and has formed the basis of a model based on the formation of OMM-IMM contact sites. However, direct evidence that this, hormone-induced, interaction between OMM and IMM membrane proteins occurs is missing. To investigate the localization, dynamics and interaction of these proteins in response to hormone treatment, we used the hormone-responsive and steroid producing MA-10 mouse tumor Leydig cells, gene expression technology and Förster resonance energy transfer (FRET) coupled to confocal microscopy. The results obtained indicate that in response to hormone stimulation, TSPO and VDAC1 in OMM and ANT1 and CYP11A1 in IMM are moving closer. Moreover, the distance between OMM and IMM is reduced suggesting the formation of OMM-IMM contact sites. This is the first time that protein movement in mitochondria is visualized in relation to hormone response leading to steroid formation. (Supported by CIHR MOP-102647)

SEARCHING FOR ELEMENTS THAT CONTROL mRNA TRANSLATION IN THE OOCYTE

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Developmentally regulated translation plays a key role in controlling gene expression during oogenesis. In particular, numerous mRNA species are translationally repressed in growing oocytes and become translationally activated during meiotic maturation. The stem-loop-binding protein (SLBP) is required for histone synthesis. *Slbp* mRNA is weakly translated in growing oocytes and becomes translationally activated during maturation. We previously showed that its translational activation required proteasomal activity, implying that an inhibitory protein must be degraded. Here, we show that a reporter mRNA bearing the 3′-untranslated region (utr) of *Slbp* becomes translationally activated during maturation and that activation requires proteasomal activity. Therefore, the inhibitory protein acts via a sequence in the *Slbp* 3′-utr. To identify this sequence, we have generated mutations in regions of the 3′-utr that are highly conserved among mammals, notably two potential PUM-binding elements and a U-rich sequence. We are currently testing the effects of these mutations on translational activation and proteasome-dependence. This study will further our knowledge of the complex mechanisms that control mRNA translation in oocytes.

MANIPULATING CLAUDIN EXPRESSION IN THE EPIBLAST

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Claudins are the molecular building blocks of tight junctions, which play critical roles in preventing the mixing of apical and basolateral proteins in the cell membrane and regulating the movement of ions and small molecules through the paracellular space. The "tightness" and permeability of the junction are determined by which members of the claudin family are present within the cell. In the Ryan lab we are investigating the role of claudin family members in embryonic patterning. We have completed a study that examined the expression of 15 claudin family members during gastrulation. Our data show both unique and overlapping claudin expression patterns across the epiblast, the cell layer that will give rise to ectoderm and neuroectoderm. The goal of my research project is to determine if the claudin expression domains are biologically significant with respect to cell fate determination.

Hypothesis: Spatially restricted zones of claudin expression confer distinct domains of ion permeability within the presumptive ectoderm and neuroectoderm cells and thereby influence embryonic patterning and morphogenesis of the ectoderm and underlying mesoderm.

Aim 1: Effect of decreasing claudin expression in the epiblast on ectodermal patterning. *C. perfringens enterotoxin* (CPE) is capable of binding to the extracellular loop of a subset of claudins causing them to be removed from the tight junction, internalized and subsequently degraded. CPE will be used to perform loss-of-function experiments in gastrulation stage chick embryos. I will overexpress the CPE in the chick ectoderm and characterize the phenotypic consequences and determine the effect on the expression of endogenous claudins.

Aim 2: Effect of claudin misexpression on patterning neural versus non-neural ectoderm. Gain-of-function experiments will be performed to determine if claudin boundaries play a role in patterning the neuroectoderm and ectoderm. I will misexpress the subset of non-neural claudins in the region of the epiblast that will become neuroectoderm and examine the embryos for morphogenetic defects that disturb gastrulation, neurulation and/or patterning of the neural tube.

CONNEXINS 46 AND 50 IN THE FOLLICULOSTELLATE CELLS OF THE ANTERIOR PITUITARY: LOCALIZATION IN THE TtT/GF CELL LINE, AND EXPRESSION DYNAMICS IN RESPONSE TO bFGF AND DURING THE MINK REPRODUCTIVE CYCLE

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Gap junction channels permit the bidirectional passage of small molecules between adjacent cells and mediate intercellular communication. The folliculostellate (FS) cells of the anterior pituitary gland communicate amongst themselves and surrounding endocrine cells through gap junctions. Connexin 43 (Cx43) is the only gap junction protein family member thus far studied in the FS cells and the TtT/GF cell, a FS cell line. We previously identified Cx46 and Cx50 in the TtT/GF cell line and mouse anterior pituitary. Here, we characterized their localization in relation to organelle and membrane domain markers and we assessed their expression in response to basic Fibroblast Growth Factor (bFGF) treatment in the TtT/GF cells. In addition, we measured Cx46 and Cx50 expression in the mink anterior pituitaries throughout the seasonal reproductive cycle. Confocal microscopy studies co-localized Cx46 with lysosomes (LAMP1), *cis*-Golgi (GM130) and *trans*-Golgi (TGN38). Cx50 was co-localized with lysosomes, *cis*- and *trans*-Golgi markers as well as with the endoplasmic reticulum (Calnexin), early endosomes (EEA1) and Flotillin1 (plasma membrane domain). Cx46 protein expression was unaffected by a 0-12 h bFGF treatment, whereas Cx50 peaked after a 4-h exposure. Cx46 and Cx50 were highly expressed in the lactating compared to non-lactating mink's anterior pituitary. However, in the adult male mink, Cx46 expression peaked in Spring, whereas Cx50 was highest in Winter. The *in vitro* and *in vivo* results suggest that Cx46 and Cx50 contribute differently to intercellular communication in the anterior pituitary. NSERC.

Mtor ACTIVITY IN THE MOUSE OVARY

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Mechanistic target of rapamycin (Mtor), a serine/threonine kinase, functions as a central regulator of cellular growth and metabolism in response to nutrient and growth-factor signals. The translational regulator Rps6kb1 is the most studied downstream effector of Mtor and its phosphorylation at T389 residue is the best indicator of Mtor activity in cells. Our previous results in mouse ovarian granulosa cells showed that the abundance of Mtor mRNA and protein was unaltered in granulosa/luteal cells during gonadotropin-stimulated follicular and corpus luteum development. In the present study, our objective was to examine if the Mtor activity in granulosa cells is induced by gonadotropins at specific stages of follicular growth and if this induction of Mtor activity is sensitive to inhibition by rapamycin. Immature female mice were superstimulated by equine chorionic gonadotropin (eCG) followed 48h later by human CG (hCG). To examine the effect of gonadotropins on Mtor activity, granulosa cells were collected by follicle puncture from the ovaries collected at 0h and 24h post-eCG (representing FSH-stimulated follicular growth) and 0h, 1h and 4h post-hCG (representing LH-stimulated preovulatory differentiation). To examine the effect of rapamycin on gonadotropin induced Mtor activity, rapamycin (10 µg/g body weight, i.p.) or vehicle was administered at 2h before eCG or hCG stimulation. There were dramatic increases in the abundance of the phosphorylated form of Rps6kb1 at 24h post-eCG as compared 0h eCG time-point, and also at 1h and 4h post-hCG compared to 0h hCG time-point. This gonadotropin induced Rps6kb1 phosphorylation was abrogated by rapamycin administration. Taken together, these data demonstrate that eCG and hCG stimulate Mtor activity in granulosa cells of follicles at specific stages of development and this gonadotropin induced activation of Mtor can be inhibited by rapamycin treatment. Overall, this pharmacologic model provides an excellent research tool to examine the key mechanisms of Mtor signaling in the regulation of ovarian functions such as follicular growth, cumulus expansion, ovulation, and steroidogenesis.

WNT PROTEIN SIGNALING AND ITS ROLE DURING THE PROCESS OF IMPLANTATION

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Successful implantation depends on precisely orchestrated and reciprocal protein signaling between the implantation competent blastocyst and the receptive uterus. Although little is known concerning the specific signaling factors involved in this process, Wnt protein signaling has been shown to significantly contribute to embryo-uterine cross-talk. Prior to implantation, Wnt proteins are secreted by the embryo and activate the Wnt signaling pathway in the luminal epithelial cells at the future site of implantation. Failure to activate this pathway severely compromises implantation. Global infertility problems are on the rise and investigating the precise role Wnt signaling plays during the process of implantation can lead towards the discovery of therapeutic targets that will ensure successful pregnancies. My research has expanded on previous microarray studies performed using pseudopregnant mice injected with Wnt7a at post-coital day 4.5. The microarray studies revealed numerous genes that were up-regulated in the luminal epithelial cells of the mouse uteri at the time implantation occurs. These genes are thought to be possible downstream target genes of the Wnt/β-catenin signaling pathway. Following up on these studies using immunohistochemistry and β-galactosidase staining, I have found that the prostaglandin I2 synthase protein, Ptgis, as well as Cyp26b1, a member of the cytochrome P450 superfamily of enzymes, could both be involved in the Wnt signaling pathway and play a role in the process of implantation. Further investigation will be conducted in order to elucidate other potential Wnt signaling factors such as Gata6, FGF1, and IGF2. Using western blotting and RT-PCR procedures, the levels of these genes and their protein counterparts will be determined at the time implantation occurs in the luminal epithelium of mouse uteri. These factors are expected to be up-regulated both in vivo and in vitro. If this is indeed the case, further studies will be conducted using anti-sense morpholino oligonucleotides to knock down the expression of target genes and determine their specific roles in the process of implantation. This research suggests that Wnt protein signaling factors, such as Ptgis and Cyp26b1, are significant components of embryo-uterine cross-talk and the proper activation of this pathway will ensure successful implantation and pregnancy.

PROLIFERATION DYNAMICS OF CARNIVORE EMBRYOS DURING ENTRY AND ESCAPE FROM OBLIGATE DIAPAUSE.

Evelyn Llerena Vargas, Pavine L.C. Lefèvre and Bruce D. Murphy. Centre de recherche en reproduction animale, Université de Montréal, St-Hyacinthe Qc J2S7C6

In many of the carnivores, including the mink (*Mustela vison*), the blastocyst is in a state of dormancy known as diapause or discontinuous embryonic development until it is reactivated by the action of prolactin secreted in response to the photoperiod of the vernal equinox. As the embryo emerges from diapause, its diameter, cell proliferation and protein synthesis all increase. However, little is known about cellular events as the embryo enters diapause nor during diapause itself. Following mating in three groups of 4 females, embryos were collected at intervals during the developmental trajectory. Collection was effected 5 days after the second mating to capture embryos at entry into diapause, while embryos from the second group, representing diapause were collected 10 days after mating. To synchronize emergence from diapause, females from third group reactivation were injected with prolactin once daily for 11 days respectively. Embryos were flushed from the uterine horns and the diameters measured as a marker for embryo activation. The embryos were then incubated in 5-ethinyl-2'-deoxyuridine (EdU) (an analog of thymidine) to establish the frequency of replication at the beginning of, during, and after diapause. To determine total cell number, embryos were stained with the nuclear dye, Hoechst 33342. Cells were counted and the ratio of replicating cells to total cells calculated. We recovered morulae with a diameter of 195.83 ± 10.93 µm and a percentage of replicating cells of 41.12 ± 5.38 at five days after mating. Some embryos were in the blastocyst stage by this time and displayed a mean diameter of 182.24 ± 11.62 µm and a percentage of replicating cells of 31.96 ± 5.70 µm. From the diapause group the blastocysts taken at day 10 post mating had a mean diameter of 230.35 ± 25.79 µm and a percentage of replicating cells of 30.47 ± 9.77. Activated blastocysts recovered following prolactin treatment showed an embryo diameter of 411.25 ± 12.46 µm and a percentage of replicating cells of 55.22 ± 4.71. During the developmental trajectory, it appeared that the trophoblastic cells replicated first, while proliferation in those of the inner cell mass occurred later. The observation that embryos in diapause continue replication was unexpected, and further analysis is underway. We conclude that proliferation decreases as the embryos pass from the morula to the blastocyst stage, but persists during diapause and that the proportion of cells that are proliferating increases rapidly with reactivation. Supported by NSERC Discovery Grant 137013 to BDM.

IS SPERM DNA DAMAGE ASSOCIATED WITH POOR IVF EMBRYO QUALITY? A SYSTEMATIC REVIEW

Naif Al-Hathal[a], Wael Jamal[b,c], Isaac Jacques Kadoch[b,c], Francois Bissonnette[b,c], Armand Zini[a].

[a]Division of Urology, Department of Surgery, McGill University, [b]Department of Obstetrics and Gynecology, University of Montreal, and [c]OVO Fertility Clinic, Montreal, Quebec, Canada.

Introduction: Sperm DNA damage is common amongst infertile men and may adversely impact natural reproduction, IUI-assisted reproduction and to a lesser degree IVF pregnancy. The objective of this study was to examine the influence of sperm DNA damage on embryo quality and/or development at IVF and ICSI.

Methods: We conducted a systematic review of studies that evaluated sperm DNA damage and embryo development and/or quality after IVF and/or ICSI.

Results: We identified 28 studies (8 IVF, 12 ICSI and 8 mixed IVF-ICSI studies) that evaluated the relationship between sperm DNA damage and embryo quality. These 28 studies evaluated 3226 treatment cycles (1033 IVF and 873 ICSI, 1320 mixed IVF-ICSI cycles) and demonstrated highly variable characteristics. In 11 of the 28 studies (1/8 IVF, 5/12 ICSI and 5/8 mixed IVF-ICSI studies), sperm DNA damage was associated with poor embryo quality and/or development, whereas the remaining 17 studies showed no relationship between sperm DNA damage and embryo quality and/or development.

Conclusions: This systematic review indicates that the evaluable studies are heterogeneous and that overall, there is no consistent relationship between sperm DNA damage and embryo quality and/or development. The data also suggest that the influence of sperm DNA damage on embryo quality/development may be more significant in ICSI compared to IVF cycles.

EFFECT OF MICROSURGICAL VARICOCELECTOMY ON HUMAN SPERM DNA INTEGRITY AND CHROMATIN COMPACTION

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Introduction: Human sperm DNA damage may adversely affect reproductive outcomes, and the spermatozoa of infertile men possess substantially more DNA damage than that of fertile men. Further to our previous study on the effect of varicocelectomy on sperm DNA integrity (DFI= DNA fragmentation index) using sperm chromatin structure assay (SCSA), the objective of this study was to further assess human sperm chromatin integrity before and after varicocelectomy by evaluating aniline blue staining (AB) in these samples.

Materials and Methods: We evaluated 20 men who underwent microsurgical varicocelectomy for clinical varicocele and 6 controls (sperm donors) at our institution. We examined standard semen parameters, sperm DNA integrity (by SCSA) and chromatin integrity (by aniline blue stain) before and after varicocelectomy and for the control group.

Results: The percentage of sperm with positive AB staining (histone retention) decreased significantly after surgery (from 13.5 to 5.4%). The %DFI (DNA fragmentation index) also decreased significantly (as previously shown). However, there were no significant relationship between AB staining and %DFI, motility or concentration. The only notable relationship was between AB staining and HDS (high DNA stainability – an index of chromatin compaction) post varicocelectomy. Furthermore, the percentage of sperm with positive AB staining was

significantly less in the control compared to varicocele patients before surgery (2.5% vs 13.5%). The %DFI also was significantly less in the control group (7% vs 20%).
Conclusion : The data show that varicocelectomy is associated with a consistent improvement in sperm DNA integrity and chromatin compaction using two different assays of sperm chromatin integrity (SCSA, Aniline Blue). However, the poor relationship between SCSA parameters (e.g. %DFI) and AB staining suggests that these assays measure different aspects of sperm function.

BREAKTHROUGHS IN REPRODUCTION AND DEVELOPMENT

Research Day 2011

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

May 4, 2011

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Li Zhang
Xiangfan Zhang
Xiaoyun Zhang
Xiang Zhou
Khaled Zohni

**Poster presentation*
***Oral presentation*
****Speaker*

NOUVELLES AVANCÉES
EN REPRODUCTION ET DÉVELOPPEMENT

Journée de recherche 2011

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

le mercredi 4 mai 2011

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**Présentateur d'une affiche*
***Présentateur oral*
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