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 2, 2012

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

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Research Day 2012

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the Human Reproduction and Development axis of the RI-MUHC

Wednesday, May 2, 2012
McGill New Residence Hall, 3625 avenue du Parc, Montreal, Quebec

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

8:45 Opening remarks: **Dr. Martine Culty**

9:00 **Dr. Jurrien Dean, Laboratory of Cellular and Developmental Biology, NIDDK, National Institutes of Health,** *"Evolving Paradigms Of Gamete Recognition In Mammalian Fertilization"* – introduced by Dr. Teruko Taketo

9:45-10:30 Oral presentations (Chairs: **Burak Özkösem and Serge McGraw**)
O-01. Yassaman Aghadazeh. "*Hormone-Induced 14-3-3γ Adaptor Protein Regulates Steroidogenic Acute Regulatory Protein Activity And Steroid Biosynthesis In MA-10 Leydig Cells*"
O-02. Serena Banh. "*Maternal Exposure Of Hydroxyurea Activates DNA Damage Response Signaling Pathways In Organogenesis Stage Mouse Embryos*"
O-03. Robert Berger. "*Increased Incidence Of External Malformations And Skeletal Variations Following Preconceptional And In Utero Exposure To Brominated Flame Retardants*"

10:30-12:00 *Health break / Poster Session*
P-01. Elie Akoury, "*NLRP7, A Nucleotide Oligomerization Domain-Like Receptor Protein, Is Required For Normal Cytokine Secretion And Co-Localizes With Golgi And The Microtubule Organizing Center*"
P-02. Shihadeh Anani, "*Investigating Polar/Apolar Cell Sorting In The 16-Cell Stage Mouse Embryo*"
P-03. EunJin Bang, "*Non-Canonical Signaling By Bone Morphogenetic Protein 2 (BMP2) Via The Type I Receptor BMPR1A (ALK3)*"
P-04. Amanda Baumholtz, "*Claudins Are Required For Neural Tube Closure*"
P-05. Erika Bezerra de Menezes, "*Influence Of Calcium On Milk Protein Aggregation And Binding Dynamics Between Binder Of Sperm (BSP) Proteins And Milk Proteins*"
P-06. Rohini Bose, "*Role Of Ubiquitin Ligase HUWE1 In Modulating Germ Cell Development And Histone Ubiquitination In The Testis*"
P-07. Anne-Marie Downey, "*Cyclophosphamide Treatment Affects MicroRNA Expression Profiles In Male Rat Germ Cells*"
P-08. Lisa Dupuis, "*Temporal Relationship Between Leptin Receptor And*

The Transcription Factor CCAAT/Enhancer-Binding Protein Beta (CEBPB) In Ovarian Granulosa Cells"
P-09. Jinjiang Fan, "*The Role Of Tspo-SINE B2 Antisense In SINE B2 RNA Toxicity Revealed By Genome Wide Expression Profiling Via Next-Generation Sequencing*"
P-10. Omar Farah, "*The Role Of Uterine Porcupine In Implantation***"**
P-11. Maira Moreno Garcia, "*Functional And Expression Analysis Of MMACHC During Mammalian Development*"
P-12. Veronica Sanchez Gonzalez, "*Live-Imaging Analysis Of Dorsal Aortae Formation In The Gastrula Mouse Embryo*"
P-13. Taghreed Heba, "*Uncovering The Function Of TMED2 During Trophoblast Differentiation***"**
P-14. Dominic Hou, "*Is TMED2 Essential In The Chorion For Normal Interaction Between The Allantois And The Chorion In Mice?*"
P-15. Claudia Lalancette, "*The Impact Of Commercial And Potential Plasticisers On Human Prostate Cell Line Functions*"
P-16. Daniel Martinez, "*In Utero Exposure To Di-(2-Ethylhexyl) Phthalate Decreases The Blood Pressure In The Adult Male Offspring*"
P-17. Thomas Nardelli, "*"Green" Compounds As Replacements For Phthalate Plasticizers*"
P-18. Geneviève Plante, "*Expression And Binding Characteristics Of Recombinant Murine Binder Of Sperm Protein Homolog 2 (BSPH2)*"
P-19. Ramesh Reddy, "*Loss Of C6ORF221 Polarization In Cells From Two Patients With Recurrent Hydatidiform Moles And Protein Truncating Mutations*"
P-20. Timothée Revil, "*The Expression And Role Of The Splicing Factors ESRP1 And ESRP2 And Their Targets During Mouse Morphogenesis*"
P-21. Marian Shafik "*The Role Of LKB1 In The Preimplantation Mouse Embryo*"
P-22. Dayananda Siddappa, "*Role Of Mtor In Testes*"
P-23. Keith Siklenka, "*Over Expression Of The Histone H3 Demethylase KDM1 In Male Germ Cells Directly Impacts Offspring Development And Is Implicated In Transgenerational Epigenetic Inheritance*"
P-24. Stella Tran, "*Impaired Fertility And Fsh Synthesis In Gonadotrope-Specific Foxl2 Knockout Mice*"
P-25. Karl Vieux, "*Expression Of Deadenylases In Growing Murine Oocytes*"
P-26. Shian Yea Wong, "*Confirmation Of Pitx2c N-Terminal Domain Interacting Proteins*"

12:00-12:30 **Dr. Florian Storch, Assistant Professor, Department of Psychiatry, Douglas Institute,** "*Circadian Clocks In Pituitary Gonadotropes"* – introduced by Dr. Daniel Bernard

12:30-1:15 Lunch (provided)

1:15-2:00 **Dr. Douglas Carrell, Director of IVF and Andrology Laboratories,**

Professor of Surgery (Urology), Obstetrics and Gynecology, and Physiology, University of Utah School of Medicine, *"Beginning To Understand The Sperm Epigenome And Its Role In Fertility"* – introduced by Dr. Jacquetta Trasler

2:00-3:00 Oral presentations (Chairs: **Jérôme Fortin and Romain Lambrot**)
O-04. Michelle Collins. "*Claudin-10 Functions On The Right Side Of Hensen's Node To Direct Left-Right Axis Patterning*"
O-05. Gurpreet Manku. "*Testicular Gonocyte Differentiation: The Importance Of The Ubiquitin Proteasome System*"
O-06. Tania Morielli. "*Oxidative Stress Promotes Redox-Dependent Protein Modifications And Impairs Motility And Capacitation In Human Spermatozoa*"
O-07. Oli Sarkar. "*Involvement Of A Variant PDGF Receptor In The Progression From Proliferation To Differentiation In Rat Testicular Neonatal Germ Cells*"

3:00-3:30 Health break

3:30-4:15 **Dr. Richard Behringer, Department of Genetics, University of Texas MD Anderson Cancer Center,** *"Reproductive Organ Formation, Homeostasis, And Regeneration"* – introduced by Dr. Yojiro Yamanaka

4:15 -4:45 **Dr. Loydie Jerome-Majewska, Assistant Professor, Departments of Human Genetics & Pediatrics, RI-MUHC Montreal Children's Hospital,** *"Expression And Requirement Of The Alternative Splicing Factors, Esrp1 And Esrp2, During Morphogenesis"* – introduced by Dr. Rima Slim

4:45-5:00 Award Presentation (best oral presentation, best poster), announcement of winner for this year's trainee representative on the CSR Executive Committee and Concluding Remarks: **Dr. Hugh Clarke**

5: 00 PM Take down posters

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

HUMAN REPRODUCTION AND DEVELOPMENT

NOUVELLES AVANCÉES EN REPRODUCTION ET DÉVELOPPEMENT

Journée de recherche 2012

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

le mercredi 2 mai 2012
McGill New Residence Hall, 3625 avenue du Parc, Montréal, Québec

8 h — Inscription et café / Installation des affiches

8 h 45 — Mot de bienvenue : **Dr. Martine Culty**

9 h — **Dr. Jurrien Dean, Laboratory of Cellular and Developmental Biology, NIDDK, National Institutes of Health,** "*Evolving Paradigms Of Gamete Recognition In Mammalian Fertilization*" – presentée par Dr. Teruko Taketo

9 h 45 — Présentations orales (Modérateurs : **Burak Özkösem and Serge McGraw**)

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O-03. Robert Berger. "*Increased Incidence Of External Malformations And Skeletal Variations Following Preconceptional And In Utero Exposure To Brominated Flame Retardants*"

10 h 30 — *Pause café / Session d'affiche*

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P-26. Shian Yea Wong, "*Confirmation Of PITX2C N-Terminal Domain Interacting Proteins*"

12 h 00 **Dr. Florian Storch, Assistant Professor, Department of Psychiatry, Douglas Institute,** "*Circadian Clocks In Pituitary Gonadotropes*" – presentée par Dr. Daniel Bernard

12 h 30 Dîner (fourni)

13 h 15 **Dr. Douglas Carrell, Director of IVF and Andrology Laboratories, Professor of Surgery (Urology), Obstetrics and Gynecology, and Physiology, University of Utah School of Medicine,** "*Beginning To Understand The Sperm*

Epigenome And Its Role In Fertility" – introduced presentée par Dr. Jacquetta Trasler

14 h 00 Présentations orales (Modérateurs : **Jérôme Fortin et Romain Lambrot**)
O-04. Michelle Collins. "*Claudin-10 Functions On The Right Side Of Hensen's Node To Direct Left-Right Axis Patterning*"
O-05. Gurpreet Manku. "*Testicular Gonocyte Differentiation: The Importance Of The Ubiquitin Proteasome System*"
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O-07. Oli Sarkar. "*Involvement Of A Variant PDGF Receptor In The Progression From Proliferation To Differentiation In Rat Testicular Neonatal Germ Cells*"

15 h Pause café

15 h 30 **Dr. Richard Behringer, Department of Genetics, University of Texas MD Anderson Cancer Center,** "*Reproductive Organ Formation, Homeostasis, And Regeneration"* – presentée par Dr. Yojiro Yamanaka

16 h 15 **Dr. Loydie Jerome-Majewska, Assistant Professor, Departments of Human Genetics & Pediatrics, RI-MUHC Montreal Children's Hospital,** *"Expression And Requirement Of The Alternative Splicing Factors, Esrp1 And Esrp2, During Morphogenesis"* – presentée par Dr. Rima Slim

16 h 45 Présentation de prix (meilleure affiche, meilleure présentation orale), l'annonce du gagnant pour le représentant des stagiaires de cette année et mot de conclusion : **Dr. Hugh Clarke**

17 h Démontage des affiches

Invited Speakers
Présentateur et présentatrices invités

Dr. Jurrien Dean, Laboratory of Cellular and Developmental Biology, NIDDK, National Institutes of Health (9:00 – 9:45 a.m.) "*Evolving Paradigms Of Gamete Recognition In Mammalian Fertilization*"

Dr. Florian Storch, Department of Psychiatry, Douglas Institute (12:00 – 12:30 p.m.) "*Circadian Clocks In Pituitary Gonadotropes*"

Dr. Douglas Carrell, Director of IVF and Andrology Laboratories, Professor of Surgery (Urology), Obstetrics and Gynecology, and Physiology, University of Utah School of Medicine (1:15 – 2:00 p.m.) "*Beginning To Understand The Sperm Epigenome And Its Role In Fertility*"

Dr. Richard Behringer, Department of Genetics, University of Texas M.D. Anderson Cancer Center (3:30 – 4:15 p.m.) "*Reproductive Organ Formation, Homeostasis, And Regeneration*"

Dr. Loydie Jerome-Majewska, Departments of Human Genetics and Pediatrics, McGill University Health Centre Research Institute (4:15 – 4:45 p.m.) "*Expression And Requirement Of The Alternative Splicing Factors, Esrp1 And Esrp2, During Morphogenesis*"

EVOLVING PARADIGMS OF GAMETE RECOGNITION IN MAMMALIAN FERTILIZATION

Jurrien Dean
Laboratory of Cellular and Developmental Biology, NIDDK
National Institutes of Health
Bethesda, Maryland 20892 USA

Biosketch: Trained in Internal Medicine at Columbia-Presbyterian Hospital in New York and in Medical Genetics at the Johns Hopkins in Baltimore, Dr. Jurrien Dean joined the NIH in 1975 as a Research Associate in Chris Anfinsen's laboratory in NIDDK. He established his independent research group there in the early 1980s and subsequently moved to the Laboratory of Cellular and Developmental Biology in NIDDK where he has served as Laboratory Chief since 1994.

Using mouse as a model system, Dr. Dean's research focuses on oocyte-specific gene expression and the role of maternal proteins in folliculogenesis, fertilization and early mouse development. Employing a broad array of technologies including molecular and cell biology as well as mouse transgenesis, these studies are designed to: 1) elucidate the molecular basis of germ- and granulosa-cell interactions; 2) explore new models of sperm-egg recognition and induction of sperm acrosome exocytosis; and 3) investigate maternal effect genes critical for cleavage-stage embryogenesis and initial establishment of cell lineages.

Education:

June 1969 A.B., Columbia College, Columbia University, New York, NY
June 1973 M.D., College of Physicians and Surgeons, Columbia University, New York, NY

Positions Held:

1973-1975 Medical Intern and Assistant Medical Resident, The Presbyterian Hospital, NY, NY
1975-1981 Research Associate, Laboratory of Chemical Biology, NIH, NIAMDD, Bethesda, MD
1977-1978 Fellow in Medical Genetics, The Johns Hopkins Hospital, Baltimore, MD
1981-1988 Investigator, Laboratory of Chemical Biology (1981-1983), Laboratory of Cellular and Developmental Biology (1983-1988), NIADDK, NIH, Bethesda, MD
1988-present Senior Investigator
1991-present Chief, Mammalian Developmental Biology Section
1994-present Chief, Laboratory of Cellular and Developmental Biology, NIDDK
National Institutes of Health, Bethesda, MD

Abstract:
For successful development, mammalian eggs must be fertilized by a single sperm. Failure of gamete recognition or absence of an effective post-fertilization block to polyspermy severely compromises reproductive fitness. The zona pellucida surrounding ovulated eggs plays a pivotal role in these biological imperatives. The mouse and human zonae pellucidae are composed of three (ZP1, ZP2, ZP3) and four (ZP1, ZP2, ZP3, ZP4) proteins, respectively. Mouse lines have been established that lack each of the three proteins. *Zp1* null females are fertile and thus, this protein cannot be essential for gamete recognition. However, *Zp2* and *Zp3* null females do not

form a zona matrix, rendering indeterminate the function of either protein in these ‘loss-of-function’ assays. Taking advantage of the observation that human sperm will not bind to mouse eggs, we also have established ’gain-of-function’ assays in which individual human zona proteins replace endogenous mouse proteins in transgenic mice. All four mouse lines are fertile, but only human ZP2 supports human sperm binding to transgenic zonae pellucidae. Following fertilization, ZP2 is cleaved by ovastacin, a cortical granule metalloendoprotease, and sperm no longer bind to the zona pellucida. Taken together, these experimental results provide new insights into the molecular role of the zona pellucida in gamete recognition and fertility in mammals.

CIRCADIAN CLOCKS IN PITUITARY GONADOTROPES

Florian Storch
Department of Psychiatry
Douglas Institute
Perry Pavilion, Room E-3214
6875 LaSalle Boulevard
Montreal, Quebec H4H 1R3

Education/Training

University of Munich B.S. 1994 Biology and Chemistry
University of Munich M.S. 1995 Biology
Max-Planck Institute for Biochemistry, Martinsried Ph.D. 1999 Biochemistry
Harvard Medical School, Boston, MA Postdoctoral 2000-2008 Neurosci./Mol. Biology
Douglas Hospital Research Centre, McGill University, Montreal, QC Assistant Professor 2008-present Mol. Neurosci./Chronobiology

Positions

2008 Assistant Professor, Douglas Hospital Research Centre, McGill University, Montreal, QC
2000-2008 Postdoctoral Fellow, Laboratory of Dr. Chuck Weitz, Department of Neurobiology, Harvard Medical School, Boston, MA

Abstract:

While body-wide alteration of circadian clock function are often times associated with reproductive deficits, the contributions of local tissue clocks to reproductive success are unknown. The pituitary gland has been shown to harbor intrinsic clock function and plays a central role in conveying reproductive signals to the gonads. Upon stimulation by GnRH, pituitary gonadotrope cells secrete luteinizing hormone (LH) which is thought to be critical for the induction of ovulation. When rodents are ovariectomized and primed with estradiol, they exhibit a daily LH surge even in constant darkness, arguing that the surge is circadian regulated. To explore the role of the intrinsic gonadotrope clock in reproductive physiology, we generated mice that carried a gonadotrope-specific disruption of the essential clock component *Bmal1* (GBmal1KO) by means of Cre-LoxP technology. GBmal1KO females proved to be fertile and did not differ from their wild-type littermates with regards to puberty onset, mating initiation, length of gestation or litter size. Vaginal cytology indicated that GBmal1KO transit through all estrous stages, however an increase of irregular and prolonged cycles was observed when compared to their wild–type littermates. While both, wild-type and GBmal1KO females were able to mount a LH surge on the afternoon of proestus, GBmal1KO animals displayed elevated LH serum levels across all cycle stages, including the time of proestrus LH surge. In contrast to GBmal1KO mice, females carrying a body-wide disruption of *Bmal1* showed a near complete loss of the LH surge on the afternoon of proestrus. Our results indicate that gonadotrope Bmal1 activity, possibly within the circadian framework, fine-tunes pituitary LH release. However, the timing of the LH surge likely relies on circadian input originating outside the pituitary.

BEGINNING TO UNDERSTAND THE SPERM EPIGENOME AND ITS ROLE IN FERTILITY

Douglas T. Carrell, Ph.D., Professor and Director of IVF, Andrology, and Reproductive Research Laboratories, University of Utah School of Medicine, Salt Lake City, UT, USA

Biosketch:

Dr. Douglas Carrell, Ph.D. is professor of Surgery (Urology), Obstetrics and Gynecology, and Physiology at the University of Utah School of Medicine. His clinical responsibilities include direction of the Andrology and IVF laboratories and is Co-Chief of the Division of Reproductive Endocrinology and Infertility. Dr. Carrell also directs a team of research associates, graduate students, and post-doctoral fellows that study genetic and epigenetic aspects of male infertility and embryogenesis. Dr. Carrell is currently co-editor of the journal *Andrology*, as well as editor of 6 recent books, including *The Genetics of Male Infertility*.

Abstract:

Sperm cells are unique in their high degree of differentiation and specialization. Included within the specialization is a unique epigenetic status, including removal of most nuclear histones and replacement with histones. Recent data has demonstrated that approximately 5-15% of sperm DNA remains bound to histones and that the retention of histones is not random, but rather reflects both a historical function as well as a programmatic function for embryogenesis. The sperm methylome also supports such a hypothesis. We have recently demonstrated that some men who consistently generate abnormal embryos during in vitro fertilization have consistent defects in the sperm epigenome, including altered histone retention and modifications. This lecture will review theses studies.

REPRODUCTIVE ORGAN FORMATION, HOMEOSTASIS, AND REGENERATION

Richard Behringer
Department of Genetics
University of Texas MD Anderson Cancer Center
1515 Holcombe Blvd.
Houston, TX 77030 USA

Biosketch:

Dr. Behringer is a mouse embryologist and expert in the genetic manipulation of the mouse, using transgenic and gene targeting methods. He is also an expert in mouse phenotype analysis. Behringer's laboratory has also pioneered 4D imaging of mouse embryos and fetal organs. In addition, he has extensive research experience in "evo-devo" and reproductive biology. More broadly, Dr. Behringer has expertise in the study of embryos from diverse mammalian species, including marsupials (opossum and wallaby), rats, bats, and whales. He also has hands-on experience with Drosophila, nematodes, hydra, sea urchins, zebrafish, Xenopus, chick, and reptiles. Thus, he has a broad appreciation of invertebrate and vertebrate embryology. Dr. Behringer has been a lecturer in the MBL *Embryology* course for the past 5 years. He has taught in the *Molecular Embryology of the Mouse* course at the Cold Spring Harbor Laboratory since 1993, serving as the course director and co-director from 1993-1996. He has also taught in developmental biology courses in Hong Kong and South America (Brazil, Argentina, and Chile). Dr. Behringer has mentored 20 graduate students to the successful completion of their Ph.D., >20 postdoctoral fellows, and >35 undergraduate researchers. Dr. Behringer has organized numerous international meetings on the topic of developmental biology, including those sponsored by the Keystone Symposia and Cold Spring Harbor Laboratory. He served for 10 years as editor of the journal *genesis:The Journal of Genetics and Development*. He is the editor of *Manipulating the Mouse Embryo, 3rd & 4th editions* and author of *Mouse Phenotypes* both by Cold Spring Harbor Laboratory Press. Dr. Behringer has also served as a member of the Board of Directors of the Society for Developmental Biology since 2009. Dr. Behringer is a Philippine American and is therefore very sensitive to the participation of mentors and trainees who are underrepresented minorities. Dr. Behringer has the broad experience and background necessary to serve as the director of the MBL *Embryology* course.

Education/Training

California State University, Northridge, CA	B.A.	1979	Biology
California State University, Northridge, CA	M.A.	1981	Biology
University of South Carolina, Columbia, SC	Ph.D.	1986	Biology

Positions

1981-1986	Predoctoral training with Dr. Michael Dewey, University of South Carolina, Columbia, SC
1986-1989	Postdoctoral training with Dr. R. L. Brinster, University of Pennsylvania, Philadelphia, PA
1989-1990	Postdoctoral training with Dr. R. D. Palmiter, University of Washington, Seattle, WA
1990-1995	Assistant Professor, University of Texas M. D. Anderson Cancer Center, Houston, TX

1995-1997	Associate Professor, University of Texas M. D. Anderson Cancer Center, Houston, TX
1997-pres.	Professor, University of Texas M. D. Anderson Cancer Center, Houston, TX
1999-2009	Editor, genesis:The Journal of Genetics and Development, Wiley & Sons, Inc., Hoboken, NJ
2000-2004	Barnts Family Professor for Cancer Research, UT MD Anderson Cancer Center, Houston, TX
2000-2008	Deputy Chairman, UT MD Anderson Cancer Center, Houston, TX
2004-pres.	Ben F. Love Chair in Cancer Research, UT MD Anderson Cancer Center, Houston, TX

Abstract:
The mammalian female reproductive organs are essential for fertility and are a common site of disease. Developmental, experimental embryological and live-imaging studies will be presented to understand the mechanisms that regulate the formation of the uterus and oviducts. In addition, the epithelial and stromal tissue compartments of the uterus have been assessed during homeostasis (estrous cycle) and regeneration (after parturition), revealing the contributions of each compartment to tissue regeneration and repair.

EXPRESSION AND REQUIREMENT OF THE ALTERNATIVE SPLICING FACTORS, ESRP1 AND ESRP2, DURING MORPHOGENESIS

Timothée Revil and Loydie Anne Jerome-Majewska
Departments of Human Genetics & Pediatrics
RI-MUHC Montreal Children's Hospital
4060 Ste-Catherine West, PT 230
Montreal, QC H3Z 2Z3

Biosketch:

Loydie A. Jerome-Majewska received her Ph.D. from the Integrated Program of Molecular Biophysics in the Department of Developmental Biology at Columbia University in 2000. Under the supervision of Dr. Virginia Papioannou Dr. Jerome-Majewska generated and characterized a mouse model of DiGeorge syndrome. She completed one postdoctoral fellowship with Dr. Papaioannou on the role of *Tbx2* and *Tbx3* on Mammary gland development. A second postdoctoral fellowship with Dr. Elizabeth Lacy focused on posterior mesoderm patterning by the extraembryonic endoderm. Since 2005, Dr. Jerome-Majewska has been an Assistant Professor in the Department of Paediatrics and an associate member of the Department of Human Genetics. Her laboratory studies the contribution of protein trafficking and alternative splicing to morphogenesis of mouse embryo and placenta. Work in her laboratory is funded by the CIHR and NSERC.

Education/Training

Wesleyan University B.A. 1991 – 1995 Biology
Columbia University M.A./M.Phil. 1995 – 1998 Developmental Biology
Columbia University Ph.D. 1998 – 2001 Developmental Biology
Columbia University Postdoctoral Fellowship 2001 - 2003
Memorial Sloan Kettering Cancer Center Postdoctoral Fellowship 2003 – 2005

Positions

2008 - Assistant Professor (Tenure Track), Department of Pediatrics, McGill University, Montreal, Canada
2008- Medical Scientist, Division of Medical Genetics, Montreal Children's Hospital of the McGill University Health Centre, McGill University, Montreal, Canada
2006 - Associate Member, Department of Human Genetics, McGill University, Montreal, Canada
2005 - 2008 Assistant Professor (Non-tenure track), Special Category, Department of Pediatrics, McGill University, Montreal, Canada

Abstract:

The evolutionarily conserved process of alternative splicing generates protein diversity in eukaryotic cells, by facilitating production of multiple mRNA isoforms from a single gene, especially in higher organisms. During mammalian embryogenesis, in a relatively short time, the genome of a single cell must generate a fetus and placenta that consists of many various cell types. We predicted that alternative splicing is one of the major mechanisms by which cell type diversity is generated in developing embryos. To test this hypothesis we used a Systems approach to identify all alternatively spliced exons during mouse development. Using strict

statistical analyses we identified over 12,000 exons that were alternatively spliced between embryonic days (E) 8.5 and E11.5, the developmental period when organs are being specified and differentiated. We examined the expression levels of known splicing factors during embryogenesis and identified two candidate genes, *Esrp1* and *Esrp2* that may coordinate expression of the exons of interest in our dataset. ESRP1 and ESRP2 are implicated in epithelial to mesenchymal (EMT) and mesenchymal to epithelial transition (MET). EMT, the process by which epithelial cell types is generated from mesenchymal cells and MET, the generation of epithelial cell types from mesenchymal cells, underlie the morphogenetic events required for normal organogenesis. Our hypothesis is that *Esrp1* and *Esrp2* are required for the normal morphogenesis of developing embryos and that abnormal alternative splicing of their targets, will result in arrested development as a consequence of abnormal EMT. We use qRT-PCR to validate differential expression of *Esrp1*, *Esrp2* and 5 targets during organogenesis. We used in situ hybridization to show tissue-specific expression of *Esrp1* and several targets in developing embryos. We are currently using siRNA and traditional homologous integration to knock this gene out during embryogenesis. Our finding suggests that the ESRP proteins regulate alternative splicing in a tissue-specific manner during organogenesis.

Oral Presentations
Présentations orales

1. Yassaman Aghazadeh (9:45 a.m.)
2. Serena Banh (10:00 a.m.)
3. Robert Berger (10:15 a.m.)

4. Michelle Collins (2:00 p.m.)
5. Gurpreet Manku (2:15 p.m.)
6. Tania Morielli (2:30 p.m.)
7. Oil Sarkar (2:45 p.m.)

HORMONE-INDUCED 14-3-3γ ADAPTOR PROTEIN REGULATES STEROIDOGENIC ACUTE REGULATORY PROTEIN ACTIVITY AND STEROID BIOSYNTHESIS IN MA-10 LEYDIG CELLS

Yasaman Aghazadeh[1], Malena B. Rone[1], Josip Blonder[2], Xiaoying Ye[2], Timothy D. Veenstra[2], D. Buck Hales[3], Martine Culty[1, 4,] and Vassilios Papadopoulos[1, 4,5].

[1]The Research Institute of the McGill University Health Centre and the Department of Medicine, McGill University, 1650 Cedar Avenue, Montreal, Quebec H3G 1A4, Canada
[2]Laboratory of Proteomics and Analytical Technologies, Advanced Technologies Program, SAIC-Frederick Inc., National Cancer Institute at Frederick, Frederick, Maryland 21702, USA
[3]Department of Physiology, Southern Illinois University School of Medicine, Carbondale, Illinois 62901, USA
[4]Department of Pharmacology and Therapeutics, McGill University, Montreal, Quebec H3G 1A4, Canada
[5]Department of Biochemistry, McGill University, Montreal, Quebec H3G 1A4, Canada

Cholesterol is the sole precursor of steroid hormones in the body. The import of cholesterol to the inner mitochondrial membrane, the rate-limiting step in steroid biosynthesis, relies on the formation of a protein complex that assembles at the outer mitochondrial membrane called the transduceosome. The transduceosome contains several mitochondrial and cytosolic components, including the steroidogenic acute regulatory protein (STAR). Human chorionic gonadotropin (hCG) induces *de novo* synthesis of STAR, a process shown to parallel maximal steroid production. In the hCG-dependent steroidogenic MA-10 mouse Leydig cell line, the 14-3-3γ protein was identified in native mitochondrial complexes by mass spectrometry and immunoblotting, and its levels increased in response to hCG treatment. The 14-3-3 proteins bind and regulate the activity of many proteins, acting via target protein activation, modification, and localization. In MA-10 cells, cAMP induces 14-3-3γ expression parallel to STAR expression. Silencing of 14-3-3γ expression potentiates hormone-induced steroidogenesis. Binding motifs of 14-3-3γ were identified in components of the transduceosome, including STAR. Immunoprecipitation studies demonstrate a hormone-dependent interaction between 14-3-3γ and STAR that coincides with reduced 14-3-3γ homodimerization. The binding site of 14-3-3γ on STAR was identified to be S194 in the STAR-related sterol-binding lipid transfer (START) domain, the site phosphorylated in response to hCG. Taken together, these results demonstrate that 14-3-3γ negatively regulates steroidogenesis by binding to S194 of STAR, thus keeping STAR in an unfolded state, unable to induce maximal steroidogenesis. Over time 14-3-3γ homodimerizes and dissociates from STAR allowing this protein to induce maximal mitochondrial steroid formation

MATERNAL EXPOSURE OF HYDROXYUREA ACTIVATES DNA DAMAGE RESPONSE SIGNALING PATHWAYS IN ORGANOGENESIS STAGE MOUSE EMBRYOS

Serena Banh and Barbara F Hales.
Department of Pharmacology and Therapeutics, McGill University, Montreal, Quebec, Canada

The administration of hydroxyurea (HU) to gestation day 9 (GD9) CD1 mice causes birth defects that include severe and characteristic damage to the fetal brain (exencephaly), appendages (clubbed feet), paws (ectro- and polydactyl), and tail (short and kinked). At embryotoxic doses, HU induces oxidative stress and a rapid onset of embryonic cell death. HU inactivation of ribonucleotide reductase has been reported to induce DNA replication stress and double-strand breaks (DSBs), thus activating DNA damage response signaling pathways. DSBs can be detected by the phosphorylation of histone H2AX on Ser 139, forming gamma H2AX (γH2AX) foci. We hypothesize that HU exposure preferentially induces the formation of γH2AX foci in malformation sensitive tissues. To investigate the spatial patterning of HU-induced DNA damage in mouse embryos during organogenesis, timed-pregnant CD1 mice were treated with vehicle or HU (low dose: 400mg/kg; high dose: 600 mg/kg) by i.p injection on GD9. Dams were euthanized at 0.5, 3, or 6h post-treatment and their embryos explanted. γH2AX foci were detected in whole embryo tissue sections using immunofluorescence and confocal microscopy. Staining was then analyzed by IMARIS 3D imaging software. Starting at 3h post-HU treatment and peaking at 6h, propidium iodide staining of the nuclei showed drastic alterations in cell morphology, including pyknotic nuclei, gross distortions of cell shape, and fragmentation of affected cells. A dramatic dose-dependent increase in γ-H2AX staining peaked at 3h in HU exposed embryos. These observations suggest that affected cells may have undergone either apoptosis or DNA repair by 6h. Whereas increases in γ-H2AX foci were detected in the rostral and caudal neuroepithelium, neural tube, and somites, all malformation-sensitive regions of the embryo, few γ-H2AX foci were detected in the heart of HU-exposed embryos, an organ that is relatively resistant to HU-induced malformations. Thus, the activation of DNA damage signaling pathways by HU in embryos during organogenesis is enhanced in malformation-sensitive tissues and associated with teratogenicity. Supported by CIHR and FRSQ.

INCREASED INCIDENCE OF EXTERNAL MALFORMATIONS AND SKELETAL VARIATIONS FOLLOWING PRECONCEPTIONAL AND IN UTERO EXPOSURE TO BROMINATED FLAME RETARDANTS

Robert G. Berger[1], Sheila R. Ernest[1], Pavine L.C. Lefèvre[1], Yi-Qian Ma[1], Michael G. Wade[3], Bernard Robaire[1,2], Barbara F. Hales[1]

[1]Department of Pharmacology and Therapeutics, McGill University, Montreal, Quebec, Canada
[2]Department of Obstetrics and Gynecology, McGill University, Montreal, Quebec, Canada
[3]Health Canada, Environmental Health Science &Research Bureau, Ottawa, Ontario, Canada

Background

Brominated flame retardants (BFRs) are used in a wide variety of consumer products and industrial goods to reduce flame ignition and slow burn rates. Elevated levels of BFRs have been positively correlated with abnormal fetal development.

Objectives

To determine if exposure of Sprague-Dawley rats during the premating and gestational periods to a BFR mixture representative of congener levels found in household dust will affect pregnancy outcome and fetal development.

Methods

Adult female rats were fed a diet containing 0, 0.75, 250 or 750 mg/kg of a mixture of BFRs based on the composition reported in North American household dust from two weeks prior to mating through to sacrifice on gestational day 20. Litter size and resorption sites were counted and fetal development and external malformations evaluated. A double staining technique to differentiate cartilage and bone was used for skeletal analysis.

Results

Mating, fertility and fecundity indices and litter sizes were not significantly affected by BFR exposure. No effects on fetal viability, sex ratio, fetal weight or crown rump lengths were detected. A significant increase in fetal external limb malformations was found after exposure to the highest doses of BFRs compared to the controls (8.8% vs 2.6%, Chi-square, $p<0.05$). Skeletal analysis showed an increase in incidence of variations in the two highest doses (Fisher Exact test, $p<0.016$). A decrease in ossification of all six sternebrae was also found following exposure at all doses (Fisher Exact $p < 0.014$).

Conclusions

Our results suggest that an environmentally-relevant mixture of BFRs can result in developmental abnormalities, including external malformations and skeletal variations, with no apparent maternal toxicity.

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CLAUDIN-10 FUNCTIONS ON THE RIGHT SIDE OF HENSEN'S NODE TO DIRECT LEFT-RIGHT AXIS PATTERNING

Michelle M. Collins[1,3] and Aimee K. Ryan[1,2,3]

[1]Department of Human Genetics, McGill University, Montreal, Quebec, Canada
[2]Department of Pediatrics, McGill University, Montreal, Quebec, Canada
[3]Research Institute of the Montreal Children's Hospital, McGill University Health Centre, Montreal, Quebec, Canada

In vertebrates, organs must be asymmetrically positioned within the body cavity to allow for normal physiological function. The origin of this asymmetry is initiated during gastrulation in an evolutionarily conserved molecular cascade. We have identified a novel molecule, *Claudin-10*, that plays a role in directing asymmetric organ positioning in the chick. Claudins are integral components of tight junctions, which act to restrict movement of ions and small molecular within the paracellular space, and interact with the actin cytoskeleton via adaptor and scaffolding proteins. Here, we report that *Claudin-10* mRNA is asymmetrically expressed on the right side of Hensen's node, the site where the left-right patterning cascade is initiated. We demonstrate that overexpression of Claudin-10 on the left side of the node, or knockdown of endogenous Claudin-10 on the right side of the node, randomizes the direction of heart-looping, the earliest morphological sign of disrupted left-right patterning. Furthermore, expression of classic left-right patterning genes *Nodal, Lefty, Pitx2c,* and *cSnR* show altered expressed in manipulated embryos, suggesting that Claudin-10 functions as an inhibitor of left-program identity. Our data also show that the PDZ-binding domain of Claudin-10 is required for its function at the node. These data demonstrate that asymmetric expression of *Claudin-10* at Hensen's node is required for normal patterning of the left-right axis.

TESTICULAR GONOCYTE DIFFERENTIATION: THE IMPORTANCE OF THE UBIQUITIN PROTEASOME SYSTEM

Gurpreet Manku[1,2], Simon Wing[1,3], Martine Culty[1,2,3]
The Research Institute of the McGill University Health Center[1], Departments of Pharmacology & Therapeutics[2] and Medicine[3], McGill University, Montreal, Quebec, Canada

The Ubiquitin Proteasome System (UPS) represents one of the main mechanisms for protein turnover and is involved in regulation of various cellular functions and signaling pathways. Alterations in UPS genes have been associated with many pathological conditions, including male infertility. In the present study, we questioned whether UPS is involved in the extensive remodeling and functional changes occurring during gonocyte differentiation to spermatogonia, a step critical for the establishment of the spermatogonial stem cell population. To begin, we identified what ubiquitin-related genes were expressed in PND3 gonocytes and PND8 spermatogonia using an Illumina gene array. We found that the transcripts of 90 ubiquitin enzymes were present in significant amounts in either germ cell type. Of these 90 genes, 5 of them were more highly expressed in PND3 gonocytes compared to PND8 spermatogonia, including the E3 ligase RNF149. We then compared gene expression in control versus differentiating gonocytes (induced by retinoic acid (RA)). Here, we saw that of these 5 genes, only RNF149 showed lower expression upon gonocyte differentiation. Thus, RNF149 appears to be downregulated in both *in vitro* and *in vivo* gonocyte differentiation. This indicates that RNF149 may play a role in gonocyte development. We then examined whether inhibition of the proteasome would have an effect on gonocyte differentiation. For this, we added the proteasome inhibitor 'Lactacystin' to isolated gonocytes in the presence or absence of RA. We saw that addition of Lactacystin inhibited RA-induced gonocyte differentiation in a dose-dependent manner, blocking the induction of the spermatogonial gene markers *Stra8* and *Dazl*. In conclusion, these studies have shown that the proteasome system is likely involved in gonocyte differentiation and have also identified RNF149 as possibly involved in this process.

OXIDATIVE STRESS PROMOTES REDOX-DEPENDENT PROTEIN MODIFICATIONS AND IMPAIRS MOTILITY AND CAPACITATION IN HUMAN SPERMATOZOA

Tania Morielli, C. O'Flaherty. Urology Research Laboratory, Urology Department, Royal Victoria Hospital, Montreal (Quebec)

Oxidative stress, caused by excessive reactive oxygen species (ROS) and/or decrease in antioxidant defences, is associated with male infertility. Oxidative stress promotes excessive redox-dependent protein modifications, such as tyrosine nitration (NO_2-Tyr) and S-glutathionylation (GSSG-R). When compared to non-treated sperm from healthy donors, we previously found that NO_2-Tyr and GSSG-R levels were higher in both spermatozoa treated with ROS, and in infertile patients. NO_2-Tyr and GSSG-R were also found to be differentially localized in sperm fractions. We hypothesize that oxidative stress promotes excessive NO_2-Tyr and GSSG-R levels, impairing sperm motility and capacitation (a process needed for the sperm to achieve fertilizing ability). Percoll washed spermatozoa from healthy donors were incubated in BWW for 30 minutes at 37^{o}C with increasing concentrations (0 to 1mM) of either H_2O_2, tert−butyl hydroperoxide, or Da-NONOate. Sperm were then washed and capacitated with 10% fetal cord serum ultrafiltrate for 3.5 h, and then incubated for 30min incubation with 2.5 uM lysophosphatidylcholine (LPC) to induce the acrosome reaction (AR). Sperm motility was assessed by CASA and CAP by determining the percentage of LPC-induced AR by FITC-pissum sativum agglutinin and the protein tyrosine phosphorylation (P-Tyr) by SDS-PAGE and immunoblotting. ROS-treated spermatozoa showed a dose dependent decrease of motility compared to controls ($p<0.05$). These spermatozoa had P-Tyr levels and %LPC-AR similar to those obtained in non-capacitated spermatozoa. In conclusion, these results suggest that excessive NO_2-Tyr and GSSG- R modified sperm proteins may be a culprit for the motility and fertilization failure observed in some conditions of male infertility. Funded by CIHR.

INVOLVEMENT OF A VARIANT PDGF RECEPTOR IN THE PROGRESSION FROM PROLIFERATION TO DIFFERENTIATION IN RAT TESTICULAR NEONATAL GERM CELLS

Oli Sarkar[1,2] and Martine Culty[1,2,3]
[1]The Research Institute of the McGill University Health Centre, Departments of Medicine[2] and Pharmacology & Therapeutics[3], McGill University, Montreal, Quebec, H3G 1A4, Canada.

Spermatogenesis depends on the formation of spermatogonial stem cells (SSCs) from their precursor cells, the gonocytes. Rat neonatal gonocytes proliferate and migrate between postnatal days 3 and 4 followed by a differentiation phase. Gonocyte proliferation is stimulated by platelet-derived growth factor BB (PDGF) and 17β-estradiol (E) in a coordinated manner via ERK1/2 activation. We previously identified a gonocyte-specific truncated variant of PDGF receptor β, V1-PDGFRβ, whose expression increased in gonocytes induced to differentiate by retinoic acid. The goal of the present study was to further examine the role of V1-PDGFRβ in gonocytes. To this end, we created a simple ID card that would indicate the state of the gonocyte by quantifying the expression of key germ cell genes including Stra8, NANOS2, DAZL, and NANOG, during PDGF+E-stimulated proliferation, retinoic acid-induced differentiation and in the presence of glial cell-derived neurotrophic factor (GDNF) and basic fibroblast growth factor 2 (FGF2). There was a down-regulation of V1-PDGFRβ during proliferation and up-regulation during differentiation, suggesting involvement of the variant in the progression of gonocytes from a proliferative to a differentiating state. Overexpression of V1-PDGFRβ stimulated gonocytes to differentiate, but addition of PDGF+E caused a decrease in differentiation markers and an increase in proliferation in V1-PDGFRβ-overexpressing cells. Furthermore, V1-PDGFRβ overexpressing gonocytes showed decreased neurogenin 3 (NGN3), a gene expressed in SSCs but not in the spermatogonia from the first spermatogenic wave. Taken together, our data shows the involvement of V1-PDGFRβ in the progression of gonocytes from a proliferative to a differentiating state and suggest that the V1-PDGFRβ-regulated transition could channel gonocytes towards the first wave of spermatogenesis.

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Poster Presentations
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1. Elie Akoury
2. Shihadeh Anani
3. EuJin Bang
4. Amanda Baumholtz
5. Erika Bezerra de Menezes
6. Rohini Bose
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19. Ramesh Reddy
20. Timothée Revil
21. Marian Shafik
22. Dayananda Siddappa
23. Keith Siklenka
24. Stella Tran
25. Karl Vieux
26. Shian Yea Wong

NLRP7, A NUCLEOTIDE OLIGOMERIZATION DOMAIN-LIKE RECEPTOR PROTEIN, IS REQUIRED FOR NORMAL CYTOKINE SECRETION AND CO-LOCALIZES WITH GOLGI AND THE MICROTUBULE ORGANIZING CENTER

Elie Akoury[1,2*], Christiane Messaed[1,2*], Ugljesa Djuric[1,2], Jibin Zeng[1,2], Maya Saleh[3], Lucy Gilbert[1,2], Muhieddine Seoud[4] , Salman Qureshi[3], and Rima Slim[1,2]

*Both authors contributed equally to this work.

[1]Departments of Human Genetics, McGill University Health Center, Montreal H3G 1A4, Canada
[2]Obstetrics and Gynecology, McGill University Health Center, Montreal H3G 1A4, Canada
[3]Department of Medicine and the Centre for the Study of Host Resistance, McGill University Health Center, Montreal H3G 1A4, Canada
[4]Department of Obstetrics and Gynecology, American University of Beirut, Beirut, Lebanon

Hydatidiform mole (HM) is a human pregnancy with hyperproliferative placenta and abnormal embryonic development. Mutations in NLRP7, a member of the nucleotide oligomerization domain (NOD)-like receptor family of proteins with roles in inflammation and apoptosis, are responsible for recurrent HMs. However, little is known about the functional role of NLRP7. Here, we demonstrate that peripheral blood mononuclear cells (PBMCs) from patients with NLRP7 mutations and rare variants secrete low levels of IL1B and TNF in response to LPS. We show that patients'cells, carrying mutations or rare variants have variable levels of increased intracellular pro-IL1B indicating that normal NLRP7 down regulates pro-IL1B synthesis in response to LPS. Using transient transfections, we confirm the role of normal NLRP7 in inhibiting pro-IL1B and demonstrate that this inhibitory function is abolished by protein truncating mutations after the Pyrin domain. Within PBMCs, NLRP7 co-localizes with the Golgi, the microtubule organizing center, and is associated with microtubules. This suggests that NLRP7 mutations may affect cytokine secretion by interfering, directly or indirectly, with their trafficking. We propose that the impaired cytokine trafficking and secretion caused by NLRP7 defects makes the patients tolerant to the growth of these earlier arrested conceptions with no fetal vessels and that the retention of these conceptions until the end of the first trimester contribute to the molar phenotype. Our data will impact our understanding of postmolar choriocarcinomas, the only allograft non-self tumors that are able to invade maternal tissues.

INVESTIGATING POLAR/APOLAR CELL SORTING IN THE 16-CELL STAGE MOUSE EMBRYO

Shihadeh Anani and Yojiro Yamanaka
Goodman Cancer Research Centre, Dept. of Human Genetics, McGill University

In the mouse embryo, the first cell differentiation occurs after the 8-16 cell (4th) division where some 8-cell blastomeres divide asymmetrically to generate outer polar and inner apolar daughter cells. The inner cells become the inner cell mass (ICM) in the blastocyst, which gives rise to the fetus and yolk-sac, and the outer cells become the trophectoderm (TE), which gives rise to the placenta. It has been thought that the outer/inner allocation of polar/apolar cells is regulated by divisional orientation during the 4th division. However, our live imaging analysis showed that many apolar cells were placed in outer positions soon after divisions and actively internalized. This suggested that the divisional orientation is not the sole driver and cell sorting is also involved in setting up the outer/inner allocation of polar/apolar cells.

In order to measure physical parameters in this sorting process, we chose to use isolated single 8-cell blastomeres as a simple model. An isolated single 8-cell blastomere can divide symmetrically or asymmetrically and forms a 16-cell couplet. When an 8-cell blastomere divided symmetrically, two daughter polar cells quickly established cell-contact after division and flattened each other. In contrast, when a blastomere divided asymmetrically, the apolar daughter cell was enveloped by the other polar daughter cell. We measured four physical parameters such as height, width (stretch) of the couplets, radius of the curvature in cell-contact, and cell-contact length. Interestingly, we found that there were four phases in the envelopment process after asymmetric divisions. First, cell-contact was established. Second, the cell-contact was curved and quickly increased to its maximum. Third, cell-contact length slowly increased without envelopment. Finally, the envelopment proceeded until the apolar cell was completely enveloped. These results were consistent with our observation in intact embryos that the boundaries between polar and apolar cells are curved towards the polar cells, while the ones between two cells of the same type are flat in intact embryos.

Our results suggest that apolar cells intrinsically have higher cortical tension than polar cells. Since the curvature formation is the first differential event leading to the envelopment process, this difference could be playing a role in setting up outer/inner allocation of polar/apolar cells.

NON-CANONICAL SIGNALING BY BONE MORPHOGENETIC PROTEIN 2 (BMP2) VIA THE TYPE I RECEPTOR BMPR1A (ALK3)

EunJin Bang[1], Ying Wang[1], Pavel Pertchenko[1], Daniel J. Bernard[1]
[1]Department of Pharmacology and Therapeutics, McGill University, Montreal, QC, Canada

Bone morphogenetic proteins (BMPs), members of the transforming growth factor (TGF) β superfamily of secreted proteins, play diverse roles in the control of reproduction and other biological functions. BMPs classically signal in cells via binding to complexes of type I and type II receptor serine/threonine kinases. Upon ligand binding, type II receptors phosphorylate type I receptors, which in turn phosphorylate the signaling proteins SMADs 1, 5, and 8. Other superfamily ligands, such as the activins and TGFβs, bind distinct receptors and thereby activate different SMAD proteins, SMADs 2 and 3. In the course of our investigation of BMP signaling mechanisms in immortalized gonadotrope cells (LβT2), we made the serendipitous and original observation that BMP2 stimulated both the canonical SMAD1/5/8 and non-canonical SMAD2/3 pathways. Here, we investigated mechanisms of non-canonical pathway activation. Using a combination of small molecule inhibitors and receptor over-expression in both homologous and heterologous cells, we demonstrated, in contrast to earlier reports, that the canonical BMP type I receptor, BMPR1A or activin receptor-like kinase 3 (ALK3), could stimulate SMAD2/3 phosphorylation and SMAD3-dependent promoter-reporter activity, in addition to its classical activation of SMAD1. This activity required the presence of a kinase-active type II receptor. Type II receptors phosphorylate type I receptors on Ser and Thr residues in their juxtamembrane domains. Results of structure-function analyses suggested that phosphorylation of specific sets of Ser and Thr residues might underlie ALK3-mediated SMAD1 versus SMAD2 phosphorylation. These data indicate that BMP signaling is more diverse and complex than previously suggested.

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CLAUDINS ARE REQUIRED FOR NEURAL TUBE CLOSURE

Amanda Baumholtz[1,3] , Michelle M. Collins[1,3], Annie Simard[3] and Aimee K. Ryan[1,2,3]
Departments of [1]Human Genetics and [2]Pediatrics, McGill University, Montreal, Quebec, Canada
[3]RI-MUHC

Neurulation is a developmental process that results in the rolling up of a flat sheet of epithelial cells into an elongated tube. Neurulation occurs in four stages: formation of the neural plate, shaping of the neural plate, bending of the neural plate and closure of the neural tube. While the process of neurulation has been extensively studied, the genes that regulate the morphogenesis of the neural tube remain poorly understood. We have completed expression analyses of 17 members of the claudin family of tight junction proteins during neurulation in chick embryos. At neurulation, claudin family members exhibited three expression patterns: uniform expression across the ectoderm, reduced expression in the neural ectoderm and enriched expression in the neural ectoderm. To determine if claudins play a role in neural tube closure, we used the C-terminal domain of *Clostridium perfringens* enterotoxin (C-CPE) to knock down claudins in the ectoderm of chick embryos at the neural plate stage. Embryos were cultured with bacterially purified C-CPE using the *ex ovo* cornish pasty method. After 20 hours, GST-treated embryos developed normally while C-CPE-treated embryos had an open neural tube, a shortened anteroposterior (AP) axis and abnormally shaped somites. Preliminary *in situ* hybridization analysis of the GST-C-CPE-treated embryos revealed that genes expressed in the neural and non-neural ectoderm have a normal expression pattern. These data suggest that claudins are required for neural tube closure and not for the initial differentiation of cells in the neural ectoderm.

INFLUENCE OF CALCIUM ON MILK PROTEIN AGGREGATION AND BINDING DYNAMICS BETWEEN BINDER OF SPERM (BSP) PROTEINS AND MILK PROTEINS

Erika Bezerra de Menezes[1,2], Marie-France Lusignan[1], Arlindo Moura[2], Puttaswamy Manjunath[1]
[1]Maisonneuve-Rosemont Hospital Research Centre and Department of Medicine, University of Montreal, Montreal, Quebec, Canada; [2]Department of Animal Science, Federal University of Ceara, Fortaleza, Ceara, Brazil.

Skimmed milk has been used as extender for sperm preservation due to its protective role against cold shock. In colloidal system, calcium is responsible for internal stability of casein micelles. Studies have shown that Binder of Sperm (BSP) proteins and their homologs from several species interact with the constituents of milk-based extenders. Thus, the aim of this study was to investigate if calcium modulates the interaction between milk proteins and BSP proteins. In order to evaluate such interaction, heated skimmed milk was incubated with seminal plasma proteins (SPP) from bull, stallion, boar and ram. Then, these solutions were loaded onto Sepharose CL-4B column previously equilibrated with buffer-C (20 mM Tris, 150 mM NaCl, 10 mM $CaCl_2$, 0.02% sodium azide, pH 7.4). The proteins were eluted and fractions were analysed by immunobloting. In parallel, SPP alone were loaded with buffer-C as controls. In the presence of calcium, casein micelles and whey protein aggregated and co-eluted together with bull, stallion and boar BSP proteins, indicating that, in these species, calcium modulates protein-protein interaction. In contrast, ram BSP proteins did not bind to milk protein aggregates, suggesting that ram BSP proteins may differ in structural organization as compared to other BSP homologs. In conclusion, our results indicate that calcium influences the formation of larger casein aggregates and the binding of BSP proteins to milk proteins. These findings are crucial to understand the mechanisms of sperm protection by diluents and for the development of novel extenders. (Supported by CAPES, Brazil and NSERC of Canada)

ROLE OF UBIQUITIN LIGASE HUWE1 IN MODULATING GERM CELL DEVELOPMENT AND HISTONE UBIQUITINATION IN THE TESTIS

Rohini Bose[1], Wenming Xu[2], Martine Culty[3], Simon Wing[1]

[1]Polypeptide Hormone Laboratory, Department of Medicine, McGill University and MUHC RI, Montreal, Quebec, Canada
[2]Laboratory of Reproductive Medicine, Sichuan University, China
[3]Department of Medicine, McGill University and MUHC RI, Montreal, Quebec, Canada

Background: Ubiquitination plays a major role in meiotic sex chromosome inactivation and histone to protamine transition during spermatogenesis. Huwe1, one of the largest HECT-domain containing E3 ubiquitin ligases, ubiquitinates all histones in vitro. It also ubiquitinates the N-myc transcriptional activator and is essential for normal differentiation of the cerebral cortex and cerebellum. I hypothesize that Huwe1 plays a similar role in regulating gene expression and development in germ cells in the testis as it does in the brain and it does so by modulating ubiquitination of histones.
Aim: To determine the effect of inactivation of Huwe1 on fertility and the developmental maturation of germ cells in the testis.
Results: DdX4-cre mice expressing recombinase in primordial germ cells were mated with female mice carrying a floxed Huwe1 allele. The Huwe1 KO males plugged the females normally but did not produce offsprings. Testicular size was reduced by 80%. Testicular morphology showed sertoli cells arranged haphazardly, leydig cell hyperplasia and barely any germ cells. Histology of the testes in postnatal day 6 and 8 KO mice showed the presence of germ cells in the tubules indicating that they undergo apoptosis at a later stage in development.
Conclusion: Huwe1 is essential for normal developmental maturation of germ cells in the testis.

CYCLOPHOSPHAMIDE TREATMENT AFFECTS MICRORNA EXPRESSION PROFILES IN MALE RAT GERM CELLS

Anne Marie Downey MSc[1], Claudia Lalancette PhD[1] and Bernard Robaire PhD[1,2]
[1]Department of Pharmacology and Therapeutics, McGill University, Montreal, QC, Canada; [2]Department of Pharmacology and Therapeutics, and Obstetrics and Gynecology, McGill University, Montreal, QC, Canada

Introduction: MicroRNA's are a class of small, non−coding RNA molecules involved in post−transcriptional regulation of gene expression. They act by complementary base pairing with the 3' un−translated region of a target messenger RNA causing either its degradation or translational repression, depending on the degree of complimentarity. Studies suggest that miRNA's likely play a role in regulating gene expression throughout spermatogenesis. Previous studies in our laboratory have shown that paternal exposure to cyclophosphamide, a chemotherapeutic and immunosuppressant, has an effect on gene expression and protein content in male rat germ cells. The mechanism by which these changes occur is not known. The goal of this study is to test whether cyclophosphamide will affect miRNA expression profiles in male rat germ cells.
Methods: Male rats were treated orally with either cyclophosphamide or saline 6 days a week for 4 weeks. Round spermatids from both control and treated rats were collected by unit gravity sedimentation using the STA−PUT method. Total RNA (including miRNA) was isolated and verified for integrity and presence of miRNA. miRNA and mRNA expression was profiled using a whole rat genome miRNA and gene expression microarrays. Data was analyzed with Genespring Software 11.5.
Results: Analysis of preliminary miRNA microarray data show that samples from treated and control animals have differential miRNA expression profiles. 208 miRNA's showed over two−fold change in expression in treated samples compared to control. 71 of these were up−regulated while 137 miRNAs were down−regulated. A target scan of up−regulated and down−regulated miRNA's revealed that these miRNA's potentially regulate many genes. Gene ontology analysis of the targets up and down regulated miRNA's revealed that target genes are involved in a multitude of processes. Gene expression microarray data show differential mRNA expression profiles between treated and control samples. Some of these mRNA's are predicted targets of the up and down regulated miRNA's.
Conclusion: Preliminary data suggests that cyclophosphamide may have an effect on miRNA expression profiles. Up and down regulated miRNA's potentially target genes that are involved in a multitude of processes. Many of these potential targets also show changes in expression. This suggests that changes in miRNA expression profiles in round spermatids could potentially have many consequences on the proper development of male gametes.

TEMPORAL RELATIONSHIP BETWEEN LEPTIN RECEPTOR AND THE TRANSCRIPTION FACTOR CCAAT/ENHANCER-BINDING PROTEIN BETA (CEBPB) IN OVARIAN GRANULOSA CELLS

Lisa Dupuis, Dayananda Siddappa and Raj Duggavathi
Department of Animal Science, McGill University

Previously using superstimulated mice, we showed that mRNA expression of leptin receptor isoforms, *LeprA* and *LeprB* was dramatically upregulated at 4h post-hCG in granulosa cells. Since leptin signaling regulates granulosa cell function, it is important to understand the regulatory mechanisms of Lepr expression. The transcription factor Cebpb has been shown to regulate Lepr expression in the Hep3B human hepatic cell line. Cebpb is expressed as three isoforms including a full length 38kDa isoform, a 35kDa LAP isoform and a truncated 20 kDa LIP isoform. A ratio between LAP and LIP isoforms potentially determines biological effects of Cebpb. The objective of the present study was to determine the expression pattern of Cebpb in granulosa cells of the ovulating follicle. Quantitative PCR was performed using superstimulated mice from which granulosa/ luteal cells were purified at specific stages of follicular/CL development. The expression of Cebpb was upregulated at 1h, 4h and 7h time-points after hCG stimulus. Analysis of protein extracted from granulosa cells of superstimulated mice during periovulatory period revealed that the full length and LIP isoforms were upregulated at 1, 4 and 7h post-hCG, while the LAP isoform was upregulated at 1 and 4 h post-hCG. The LAP:LIP ratio was higher at 1 and 4h post-hCG, while it was low at 7h post-hCG. These results demonstrate that Cebpb is induced by hCG prior to transcriptional induction of Lepr suggesting that Cebpb may act as a regulator of Lepr in granulosa cells of the ovulating follicle.

THE ROLE OF *TSPO*-SINE B2 ANTISENSE IN SINE B2 RNA TOXICITY REVEALED BY GENOME WIDE EXPRESSION PROFILING *VIA* NEXT-GENERATION SEQUENCING

Jinjiang Fan[1], Daniel B. Martinez-Arguelles[1] and Vassilios Papadopoulos[1,2]
[1]The Research Institute of the McGill University Health Center and [2]Departments of Medicine, Biochemistry, and Pharmacology & Therapeutics, McGill University, Montréal, Québec H3A 1A4, Canada

Natural antisense transcripts (NATs) are an essential feature of many eukaryotic genomes, including human and mouse. Short interspersed repetitive element B2 (SINE B2), previously known as "junk DNA", in the mouse genome encodes a mobile RNA polymerase II (pol II) promoter and drives transcription of short antisense transcripts corresponding to these elements. Traditionally, the length of these antisense transcripts has been assumed to be limited. We recently reported the presence of a SINE B2-mediated translocator protein (18-kDa) *Tspo*-NAT overlapping with *Tspo* exon 3 and functioning as a NAT to control *Tspo* gene expression and function in MA-10 mouse Leydig cell steroidogenesis (Biol Reprod 2012; PMID 22378763). Using *in silico* screening, pilot experimental verification, and next-generation sequencing analysis, we now provide evidence that SINE B2 elements drive the genome-wide transcription of the SINE B2-mediated long antisense transcripts. A total of 96,862 sequences with a length of over 130 bp were generated, representing 27.4% of the SINE B2 elements in the mouse genome. The identification of SINE B2-mediated and/or -containing transcripts in mouse dbESTs further implied the potential role of these elements in the regulation of gene transcription. Strand-specific Northern hybridization analyses demonstrated that the SINE B2-antisense transcripts (SINE B2-AS) play a role in the self-regulation of SINE B2-sense transcript (SINE B2-S) expression. *Dicer1* functions as a ribonuclease involved in miRNA processing to produce active small RNAs that repress gene expression. Knockdown of *Dicer1* in MA-10 cells using *Dicer1*-AS, followed by comparative quantitation of the RNA expression from a total of 106.2 million reads in *Dicer1*-AS treated cells with the 61.9 million reads in the control cells, indicated that SINE B2-AS RNAs are networked with the miRNA system as a "transcriptome buffer". Moreover, the data obtained indicated that knocking down *Dicer1* in MA-10 cells resulted in increased expression of SINE B2-driven *Tspo*-NAT as well as *Tspo* SINE B2-S, located just after the first *Tspo* exon, with 56.3 more base pair quantitation counts, which refers to how many bases from each read overlaps in that specific region. These findings are inversely correlated to the reduced *Tspo* levels, with 2.4 less base pair quantitation counts in the first exon than that of the control. Taken together these results indicate that the overexpressed *Tspo* SINE B2-S in the first intron blocks *Tspo* transcription, in addition to our previous report that a SINE B2-driven *Tspo*-NAT directly targets exon3. These data identifies the mitochondrial TSPO as a DICER1 target gene. Because TSPO, in addition to its role in cholesterol transport in steroidogenic cells, is also involved in the regulation of the mitochondrial permeability transition pore, apoptosis and stress response, these results indicate a new mechanism for SINE B2-S mediated toxicity in cells.

THE ROLE OF UTERINE PORCUPINE IN IMPLANTATION

Omar Farah, Daniel Dufort

The Porcupine gene encodes a protein of the conserved O-acyl transferase superfamily required for the lipid modification of the Wnt proteins. Palmitoylation of the Wnt proteins in the endoplasmic reticulum is required for their secretion. The secretion of various WNTs, by both the uterus and the embryo, has been found to be necessary in creating a cross talk between the embryo and the uterus in order to facilitate implantation. Inhibition of some of these Wnt genes has been shown to reduce fertility and implantation frequency. In order to fully establish the role of Wnts during implantation, it is necessary to eliminate the expression of all Wnt genes. Deletion of the porcupine gene will result in the cell's inability to secrete WNTs, therefore compromising their extra-cellular function. A uterine specific deletion of Porcupine using a progesterone receptor-Cre (PR-Cre) system has been established in order to address this issue.

Preliminary results indicate that wild-type porcupine is mainly expressed in the luminal and glandular epithelium of the uterus. The expression level, at the transcriptional level, does not appear to change during the window of implantation. We have generated heterozygous uterine specific deleted Porcupine and have found that although these mice appear to be cycling regularly, most of these heterozygous females are sterile and are not able to reproduce. In addition, some of the heterozygous females display an abnormal morphological phenotype of the uterus. The uterus appears to have large multiple cavities in the stroma, sometimes larger than the lumen itself. The cells lining these cavities exhibit epithelial properties similar to those of the luminal and glandular epithelium. We hypothesize that the knockout females, we are currently working on, will display a similar phenotype to that of the heterozygous females.

FUNCTIONAL AND EXPRESSION ANALYSIS OF MMACHC DURING MAMMALIAN DEVELOPMENT

Maira A Moreno Garcia[a]; Mihaela Pupavac[a], David S Rosenblatt[ab] and Loydie Jerome-Majewska[ab]

[a]Department of Human Genetics, McGill University, Montreal, Quebec, Canada H3G 1B1

[b]Department of Pediatrics, McGill University, Montreal, Quebec, Canada H3Z 2Z3

Deficiencies of vitamin B_{12} or defects in its metabolism are known as inborn errors of metabolism. A series of inborn errors of vitamin B_{12} metabolism have been identified, designated *cblA-cblG*. The *cblC* disorder (OMIM 277400), is the most frequent inborn error of vitamin B_{12} metabolism and is due to mutation in MMACHC. Patients with this disease have hematological, neurological, and ophthalmological findings, along with developmental delay. To elucidate the function of MMACHC and examine the expression pattern of MMACHC during mouse embryogenesis, we studied a gene-trap mouse line with a functional disruption of the *MMACHC* gene and we determined sites of gene expression at 11.5 days post conception in WT mouse by *in situ* hybridization. *In situ* hybridization analysis showed that *MMACHC* gene has a specific and restricted expression pattern during organogenesis in the mouse embryo. The gene-trap mouse line showed that heterozygous mutants were fertile and viable. However, homozygous embryos were only obtained at embryonic day 3.5. At the same time, we found by western blotting, that heterozygous mutants at E11.5 had decreased levels of MMACHC proteins and decrease in proliferation compared with the wild-type mice. Our results highlight that murine MMACHC is essential for early embryonic development.

LIVE IMAGING ANALYSIS OF DORSAL AORTAE FORMATION IN THE GASTRULA MOUSE EMBRYO

Veronica Sanchez, Yojiro Yamanaka
Human Genetics Department, McGill University, Montreal, Quebec, Canada

Blood vessel formation, termed vasculogenesis, is essential for early embryonic development. The first vasculogenesis occurs prior to blood circulation and results in formation of the extra-embryonic yolk sac vasculature and the intra-embryonic dorsal aortae (DA). Endothelial cells (EC), derived from progenitor cells called angioblast, are responsible for structuring blood vessels. Flk1 is expressed in angioblast/endothelial cells and it is essential for formation of the vascular system. It mediates cell migration, proliferation and differentiation. Homozygous null Flk1 mutant embryos cannot form the both embryonic and extraembryonic vascular systems. It is not clear if they share the same origin. It is our intent to analyze the formation of the DA to identify their angioblast origin. To visualize angioblast/endothelial cells, we use the Flk-GFP mouse reporter line, which is a GFP knock-in mouse to the endogenous Flk1 allele. Live-imaging analysis of E7.5 gastrula embryos showed the appearance of a small population of Flk1-GFP positive cells actively migrating from the extra-embryonic region into the embryonic region. During migration, the cells start aggregating and luminal formation is initiated. Embryonic DA forms as these small luminal aggregates line in the embryonic region and form a distinct tubular structure. At this point, the GFP signal in some cells becomes stronger. Interestingly, during this process we identified that there are two distinct levels of Flk1-GFP expression, high and low. Only Flk1-GFP high cells contribute to the formation of the DA. Further studies are required to figure out the identity and the roles of Flk-GFP low cells.

UNCOVERING THE FUNCTION OF TMED2 DURING TROPHOBLAST DIFFERENTIATION

Taghreed Heba, Abeer Zakariyah, Loydie A. Jerome-Majewska

Transmembrane emp24 domain trafficking protein, (TMED2) is a member of the p24 family of proteins involved in vesicle transport between the ER and Golgi. During vesicular transport between the ER and Golgi p24 proteins function as receptors for both cargos and coat proteins. Our group showed that *Tmed2* is required for normal embryo and placental development in mouse and that syncytiotrophoblast cells of the mouse labyrinth placenta failed to differentiate in homozygous mutant embryos. In human placenta, we showed expression of TMED2 between 5.5 and 40 weeks of gestation in all trophoblast cell types. We noted that early in gestation TMED2 was more highly expressed in cytotrophoblast cells versus syncytiotrophoblast. Surprisingly, we found that TMED2 was more highly expressed in a choriocarcinoma cell line, BeWo, which can be induced to differentiate and form syncytiotrophoblast when compared to the JEG-3 cell line, which does not fuse to form syncytiotrophoblast. We hypothesized that TMED2 is required for fusion of trophoblast cells during syncytiotrophoblast differentiation. To test this hypothesis we are examining the function of TMED2 during trophoblast differentiation of BeWo and Jeg-3 cell lines. We will show our plans to ectopically express TMED2 in Jeg-3 cells and to knockdown TMED2 expression in BeWo choriocarcinoma cells with shRNA. Our work suggests that TMED2 is required for trafficking cargoes that are essential for placental development.

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

W. Hou[1], D. Sarikaya[1,3], L. Jerome-Majewska[1,2]

1) Department of Human Genetics, McGill University, Montreal, QC, Canada; 2) Montreal Children Hospital Research Institute, Montreal, QC, Canada; 3) Organismic and Evolutionary Biology Department, Harvard University, Cambridge, Massachusetts, USA.

Keywords: placenta, labyrinth layer, chorioallantoic attachment

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. We hypothesized that TMED2 is essential in the chorion or allantois 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 and immunohistochemistry to confirm the chorioallantoic attachment event and to monitor development of labyrinth layer in the chimeric explants.We will then use combinations of wild type and *Tmed2* null chorion and allantois in these ex-vivo cultures to follow branching morphogenesis in the chorion. Our work will provide insight into the contribution of placental-specific vesicular transport by TMED2 to labyrinth layer morphogenesis. Ultimately we will identify novel mechanisms that may be implicated in the prediction and treatment of placental diseases such as EPL and IUGR.

THE IMPACT OF COMMERCIAL AND POTENTIAL PLASTICISERS ON HUMAN PROSTATE CELL LINE FUNCTIONS

Claudia Lalancette and Bernard Robaire, Departments of Pharmacology and Therapeutics and Obstetrics and Gynecology, McGill University, Canada

Animal studies showed that the current commercial plasticisers, especially phthalates, have detrimental effects on the development of the male reproductive system. Although the main focus has been on testicular development, animal and cell culture models show evidence that the prostate is also a target, but the observations are limited. As part of a multi-disciplinary project with the aim to identify safer plasticisers, we examined the impact of commercial plasticisers, as well as potential replacements, on the function of human prostate cell lines. The main objective of this study was to investigate the impact of current and potentially new plasticizers on human prostate cell lines. Human prostate cell lines LNCaP, DU145, PC3 and PNT1A were exposed to commercial plasticisers currently in use, such as Di(2-ethylhexyl) phthalate (DEHP) and 1,2-cyclohexane dicarboxylic acid diisononyl ester (Hexamoll DINCH), as well as additional compounds shown to have plasticising qualities (dibenzoate- and succinate-based). Cell viability was measured using the MTT assay. Gene expression was compared using real-time PCR. Protein expression or secretion was evaluated using Western blot. The human prostate cell lines showed different sensitivities upon exposure to the tested plasticisers. None of the cell lines were affected by succinates. Hexamoll DINCH induced a 20% decrease in cell survival for the DU145 and PC3 cell lines. Only the DU145 cell line showed a marked decrease in cell survival in the presence of dibenzoates. DEHP induced a 40% decrease in cell viability for DU145 and a slight decrease for LNCaP. Interestingly, gene expression for kalikrein 3 (PSA) in LNCaP was decreased in the presence of either phthalate or dibenzoates. For the DU145 cell line, there was an increase in the expression of the glucocorticoid receptor after incubation in the presence of dibenzoates. We conclude that succinate-based plasticisers may be safe alternatives to the current commercial plasticisers. The differences in response based on the cell lines tested suggest that when possible, multiple cell lines for the organ of interest be tested. The cell line sensitivities to the tested compounds also suggest that different pathways are involved. We are currently working on identifying those pathways that make the prostate cell lines responsive to different compounds. These studies were funded by a Team Grant from the Canadian Institutes of Health Research.

IN UTERO EXPOSURE TO DI-(2-ETHYLHEXYL) PHTHALATE DECREASES THE BLOOD PRESSURE IN THE ADULT MALE OFFSPRING

Martinez–Arguelles DB [1,2],McIntosh M [1,3], Rohlicek C [1,3], Culty M,[1,2,4] Zirkin BR[5], and Papadopoulos V[1,2,4,5,6]

[1]The Research Institute of the McGill University Health Centre, Montreal, Quebec, H3G 1A4, Canada

[2]Department of Medicine, McGill University, Montreal, Quebec, H3G 1A4, Canada

[3]Department of Pediatrics, McGill University, Montreal, Quebec, H3G 1A4, Canada

[4]Pharmacology & Therapeutics, McGill University, Montreal, Quebec, H3G 1A4, Canada

[5]Department of Biochemistry and Molecular Biology, Johns Hopkins University Bloomberg School of Public Health, Baltimore, Maryland 21205, USA

[6]Pharmacology & Therapeutics, McGill University, Montreal, Quebec, H3G 1A4, Canada

Background Di-(2-ethylhexyl) phthalate (DEHP) used in the industry to add flexibility to PVC polymer, is ubiquitously found in the environment and there is evidence of prenatal, perinatal, and early life human exposure. In utero exposure to DEHP decreases circulating testosterone levels in the adult rat. In addition, DEHP reduces the expression of the angiotensin II receptors in the adrenal resulting in decreased circulating aldosterone levels. Aldosterone acting through MR, present in Leydig cells of the testis, blocks androgen production and this could be a mechanism mediating the antiandrogenic effects of fetal exposure to DEHP in the adult. However, the main role of aldosterone is to control water and electrolyte balance and a chronic decrease in mineralocorticoid levels can also impact blood pressure.

Objectives Determine the effects of in utero exposure to DEHP in blood pressure in the young adult and elderly rat.

Methods Sprague-Dawley pregnant dams were exposed from gestational day 14 until birth to 300 mg DEHP/kg/day. Blood pressure, heart rate, and activity data were collected using an intra-aortal transmitter in the male offspring at postnatal day (PND) 60 and PND200. A low- and high-salt diet was used to challenge the animals at PND200.

Results In utero exposure to DEHP reduced heart rate and activity at PND60. At PND200, systolic, diastolic pressure, and activity were reduced in response to DEHP exposure.

Conclusions This is the first evidence showing that in utero exposure to DEHP has cardiovascular and behavioral effects in the adult male offspring that worsen over time.

“GREEN” COMPOUNDS AS REPLACEMENTS FOR PHTHALATE PLASTICIZERS

Thomas Nardelli[1], Claudia Lalancette[1], Hanno Erythropel[3], Milan Maric[3], David Cooper[3], Richard Leask[3], and Bernard Robaire[1,2]

Departments of Pharmacology and Therapeutics[1], Obstetrics and Gynecology[2], and Chemical Engineering[3] McGill University

Male reproductive health has been steadily declining over the past few decades. While the cause of this trend is subject to debate, endocrine disrupting compounds appear to be a contributing factor. [1] Recent work in animal models suggests phthalate plasticizers can behave as endocrine disruptors by blocking androgen signaling independently of the androgen receptor. [2] This impairment leads to a variety of developmental abnormalities collectively termed the “testicular dysgenesis syndrome”. In light of this information, regulatory agencies are moving to phase out the use of phthalates in favor of safer “green” compounds. Finding a replacement is a multi-dimensional problem because the alternative must meet the demands of consumers, industry, and government. An ideal candidate should be able to function as a plasticizer, be cost effective, and have a small environmental footprint.

Working with the Department of Chemical Engineering at McGill University, we are testing the safety of several families of compounds that are: (1) commercially available (DINCH, DEHP), (2) variants of the phthalate structure (succinate, maleate, fumarate series), or (3) a novel series of dibenzoate plasticizers that do not contain ether linkages. Our experiments were designed to follow a paradigm presented by the National Academy of Sciences in their book *“Toxicity Testing in the 21st Century: A Vision and a Strategy”*. Using two immortalized mouse Sertoli cell lines (MSC-1, TM4) and one human immortalized hepatic cell line (HepG2) we assessed toxicity of the parent compounds using the MTT Assay. The MTT Assay is a colorimetric assay that reduces 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide to small purple formazan crystals in living cells. By quantifying the extent of this reaction, it provides an indirect measure of cell viability that is compatible with high-throughput applications.

Our preliminary work suggests the succinate and dibenzoate series are the best candidates to pursue since they deviate minimally from control values (except at very high concentrations). Furthermore, 1,6 hexanediol dibenzoate readily biodegrades [3], is easy to synthesize, and is a good plasticizer. Our cell lines appear to be sensitive to the presence of maleate plasticizers. While not significant, there is a decreasing trend in cell viability starting at 10^{-6}M that continues until our highest dose of 10^{-4}M. These studies were funded by CIHR.

EXPRESSION AND BINDING CHARACTERISTICS OF RECOMBINANT MURINE BINDER OF SPERM PROTEIN HOMOLOG 2 (BSPH2)

[1,2]Geneviève Plante, [2,3]Claude Lazure and [1,2]Puttaswamy Manjunath

[1]Maisonneuve-Rosemont Hospital Research Centre, Montréal, Québec, Canada
[2]Faculty of Medecine, Université de Montréal, Montréal, Québec, Canada.
[3]Institut de Recherche Clinique de Montréal (IRCM), Montréal, Québec, Canada

Capacitation is a maturation step that is deemed to be essential for sperm to fertilize oocytes. A family of proteins, the Binder of Sperm (BSP), are known to bind choline phospholipids on sperm membranes 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. BSP-homologous genes have also been identified in humans, primates and rodents. Interestingly, in these species BSP genes are expressed in epididymis rather than in seminal vesicles. BSP genes in human (*BSPH1)* and mice (*Bsph1)* have been cloned and characterized. Recent studies have demonstrated that a recombinant murine BSPH1 could bind to heparin, gelatine, phosphatidylcholine-liposomes and promote sperm capacitation. The objective of the current study was to determine if BSPH2, the other murine BSP protein, plays a similar role in sperm function. To do so, we produced recombinant BSPH2 (rec-BSPH2) using Rosetta-gami B(DE3)pLysS cells to help with the folding of the proteins in combination with a pET32a vector which adds a thioredoxin tag to the expressed proteins making them more soluble. We used an immobilized metal ion affinity chromatography technique and obtained a good yield of pure protein. The tag-free BSPH2 was obtained by treatment with enterokinase and the identity of BSPH2 was confirmed by N-terminal sequencing and mass spectrometry. The rec-BSPH2 (with or without tag) cross-reacted with anti-BSPH1 antibodies. The preliminary data suggests that the rec-BSPH2 can bind to heparin similar to BSPH1. The availability of the tag-free protein should aid in establishing its function(s). *(Supported by NSERC, CIHR and FESP of university of Montréal)*

LOSS OF C6ORF221 POLARIZATION IN CELLS FROM TWO PATIENTS WITH RECURRENT HYDATIDIFORM MOLES AND PROTEIN TRUNCATING MUTATIONS

Ramesh Reddy[1,2*], Elie Akoury[1,2*], Omar A. Abdul-Rahman[3], William P. Daley[4], Hanene Landolsi[5], Rosemary Fisher[6], Isabelle Touitou[7], Rima Slim [1,2,*]

*The authors contributed equally to this work.

[1]Departments of Human Genetics and [2]Obstetrics and Gynaecology, McGill University Health Centre, Montreal H3G 1A4, Canada.
[3]Department of Pediatrics, [4]Department of Pathology, University of Mississippi Medical Center, Jackson 39211, Mississippi, US.
[5] Laboratoire d'anatomie et de cytologie pathologiques, Centre Hospitalier Universitaire, Farhat Hached, Rue Ibn Eljazzar, Sousse 4000, Tunisia.
[6] Institute of Reproductive and Developmental Biology, Imperial College London SW7 2AZ, UK.
[7]Unité médicale des maladies auto-inflammatoires, Laboratoire de génétique, Hôpital Arnaud de Villeneuve, CHRU Montpellier-Université Montpellier 1, INSERM U844, Montpellier, France.

Hydatidiform mole is a human pregnancy with abnormal embryonic development and hyperproliferation of trophoblastic cells. Two causal genes, NLRP7 and C6orf221, responsible for this condition have been described. NLRP7 is the major gene with sixty-two reported mutations to date. C6orf221 is a recently identified minor gene, in which three mutations have been reported. In this study, we sequenced C6orf221 in a total of 89 patients with recurrent moles and reproductive wastage. We identified two patients with homozygous protein truncating mutations, a novel 4-bp deletion, c.298_302delTCAA, p.Ile100Argfs*2 in patient 481, and a previously described mutation, c.322_325delGACT, p.Asp108Ilefs*30 in patient 654. Our RT-PCR results in these two patients show that there is no Non-sense mediated mRNA decay in these patients. Within hematopoietic cells, we show that normal C6orf221 protein is polar and co-localises with NLRP7 to the Golgi apparatus and microtubule organizing center. In addition, we show that cells from our two patients with homozygous protein-truncating mutations have defective C6orf221 polarization. Our data delineate further the functional roles of C6orf221 and NLRP7 in the pathology of recurrent moles and highlight the presence of several founder mutations and non-synonymous variants in these two genes in several populations indicating positive selection and adaptation.

THE EXPRESSION AND ROLE OF THE SPLICING FACTORS ESRP1 AND ESRP2 AND THEIR TARGETS DURING MOUSE MORPHOGENESIS

Timothée Revil and Loydie Anne Jerome-Majewska

The development of an embryo from oocyte to a fully formed embryo requires highly regulated gene expression changes. Using a systems approach, we have shown that, in addition to these changes in whole gene expression, there are over 12,000 alternative splicing events that are regulated during embryogenesis. Upon analysis of the expression levels of known splicing factors, we identified two splicing factors, ESRP1 and ESRP2, whose expression correlated with the inclusion of a subset of alternative exons. These two proteins have been shown to regulate alternative splicing of targets potentially implicated in epithelial to mesenchymal transitions (EMT) in human cell lines. We have studied the localization of the expression of these genes during mouse embryogenesis using *in situ* hybridization at stages E6.5 to E10.5. We show that *Esrp1* is broadly expressed early in development, and then gradually restricted to the endoderm and ectoderm of certain developing structures. We are currently performing wholemount *in situs* for putative target alternative exons as well as section *in situs*. In order to determine the importance of these genes during development, we are also performing knockdown experiments using siRNAs on blastocysts which will be re-injected into pseudo-pregnant females and analyzed. Finally, we are in the process of creating a conditional knockout mouse for *Esrp1*.

THE ROLE OF LKB1 IN THE PREIMPLANTATION MOUSE EMBRYO

Marian Shafik and Yojiro Yamanaka
Human Genetics Department, McGill University, Montreal, Quebec, Canada

Lkb1, a tumor suppressor gene, is associated with a rare autosomal dominant disorder known as Peutz-Jeghers Syndrome (PJS), characterized by the formation of benign gastrointestinal polyps and increased risk for malignant tumors.

It has been shown that Lkb1 is involved in various cellular events such cell cycle regulation, cell polarity formation and epithelial morphogenesis, and energy metabolism. The role of Lkb1 in development has been examined in *Lkb1* KO mice. Interestingly, homozygous mutant embryos survive up to E9.5 and die due to vasculogenesis defects. Although cell polarity and epithelial morphogenesis play important roles in cell allocation and lineage specification in preimplantation embryos, no clear defect in these processes has been observed.

We hypothesize that the lack of early phenotypes in the *Lkb1* mutant embryo is due to the maternal load of Lkb1 protein and mRNA which is sufficient for the embryo to go through the early stages of development.

To test this, we generated maternal/zygotic null *Lkb1* embryos using the oocyte-specific Cre line, *ZP3*-Cre, with the floxed *Lkb1* conditional KO mouse line.

Interestingly, both maternal/zygotic-deleted homozygous (*MZLkb1-/-)* embryos and maternal-deleted heterozygous (*MLkb1+/-)* embryos established epithelial layers at E3.5 and E4.5 with clear apico-basal polarity. Although the first three cell lineages, trophectoderm, epiblast and primitive endoderm were formed, we found a bias towards a particular cell fate at E3.5 in both *MLkb1+/-* and *MZLkb1-/-* embryos.

Also, we found changes in cell allocation and the morphology of the blastocyst ICM. We are currently measuring these defects in a quantitative manner.

Taken together, our results suggest that a maternal supply of Lkb1 directs aspects of development in the early mouse embryo development.

ROLE OF MTOR IN TESTES

Dayananda Siddappa[1], Tamara Cohen[1], Jennifer Baird[1], Bernardo Gesperin[2], Raj Duggavathi[1]
[1] Department of Animal Science, McGill University, Ste-Anne-de-Bellevue, Canada
[2] Clinica de Grandes Animais, Universidade Federal de Santa Maria, Santa Maria, Brazil

Mechanistic-target-of-rapamycin (Mtor), a serine-threonine kinase, regulates cell proliferation and growth in response to external stimuli including hormones, growth factors and nutrients. In adult mouse testes, Mtor protein is highly expressed in somatic cells (sertoli and leydig cells) and in also spermatogonial cells. To understand the importance of Mtor signaling in testicular somatic cells, we developed Mtor-conditional knockout (cKO) mice by breeding Mtor-floxed mice with Amhr2-cre mice. Control (CON) and cKO mice (N=3-10/genotype) were evaluated for reproductive parameters at days 21, 35, 90 and 180 of age. Testis weight was not different between the genotypes until d 35**,** but there was significant reduction in cKO mice compared to CON mice at the age of 90 d and 180 d. Reduced size of cKO testes at d 180 was associated with markedly lower total sperm count and sperm density. Motility of sperms obtained from cauda epididymis was similar (70-80%) between CON and cKO mice. However, sperms from 180 d old cKO mice were immotile. In addition, the percentage of morphologically abnormal sperms was significantly higher in cKO mice at both d 90 and d 180. By d 180 cKO mice epididymis showed severe depletion or absence of sperms in cauda. Microscopic examination of testes of 180 d old mice revealed that cKO testes were replete with severe histological abnormalities including diffused vacuolation of sertoli cells, leydig cell hyperplasia, germ cell hypoplasia and dying round spermatids. Taken together, these data show that cKO mice have sever testicular dysfunction by the age of 6 months. Thus, we conclude that Mtor in somatic cells of the testis is required for normal spermatogenesis and the absence of which results in progressive infertility.

OVER EXPRESSION OF THE HISTONE H3 DEMETHYLASE KDM1 IN MALE GERM CELLS DIRECTLY IMPACTS OFFSPRING DEVELOPMENT AND IS IMPLICATED IN TRANSGENERATIONAL EPIGENETIC INHERITANCE.

Keith Siklenka[1], Maren Godmann[2], Romain Lambrot[2], Christine Lafleur[2], Mathew Sudermann[3], Mike Hallett[3] and Antoine Peters[4] and Sarah Kimmins[1,2]

[1]Department of Pharmacology and Therapeutics, McGill University, Montreal, Quebec, Canada.
[2]Department of Animal Science, McGill University, Montreal, Quebec, Canada.
[3]McGill Centre for Bioinformatics, McGill University, Montreal, Quebec, Canada.
[4] Friedrich Miescher Institute for Biomedical Research (FMI), CH-4058, Basel, Switzerland

Histone modifications such as histone H3 lysine 4 (H3-K4) and lysine 9 methylation (H3-K9), are present at the time of epigenetic reprogramming in spermatogenesis (Godmann et al., 2007; Biology of Reproduction), and have been localized in sperm to genes implicated in embryonic development (Hammoud et al., 2009; Nature). Lysine Specific Demethylase 1 (KDM1) is endogenously expressed in the testis and functions to remove H3 lysine 4 (K4) and lysine 9 (K9) mono- and di-methylation during spermatogenesis. Characterization of two heterozygous transgenic C57bl6 mouse lines, which overexpress human KDM1 specifically in the testis, has revealed that 39% of pups sired by transgenic males show developmental abnormalities while 36% die before postnatal day 21. Abnormal development and reduced survivability was shown to increase with each successive generation. Importantly, these effects were not limited to offspring sired directly by transgenics, but have been inherited by pups sired by wild-type descendants of transgenic fathers (termed EI offspring for epigenetic inheritance). Analysis of litters sired by EI F1 (n=18) and EI F2 (n=30) at gestation day 18.5 showed increased abnormal pups as well as pre- and post-implantation loss when compared to control litters (n=19). Analysis of the sperm epigenome from TG and EI males by MeDIP-Seq and ChIP-Seq revealed widespread alterations in DNA and histone methylation. Ongoing analysis of EI F3 will aim to determine the persistence of the observed transgenerational effects on offspring and identify DNA sequences and corresponding epigenetic modification implicated in transgenerational epigenetic inheritance.

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IMPAIRED FERTILITY AND FSH SYNTHESIS IN GONADOTROPE-SPECIFIC *FOXL2* KNOCKOUT MICE

Stella Tran[1], Xiang Zhou[1], Christine Lafleur[2], Sarah Kimmins[1,2], Derek Boerboom[3], Ulrich Boehm[4], Mathias Treier[5], Daniel J. Bernard[1]

Departments of Pharmacology and Therapeutics[1] and Animal Science[2], McGill University; [3]Department of Veterinary Biomedicine, University of Montreal; [4]Center for Molecular Neurobiology, Hamburg; [5]Max-Delbrück Center for Molecular Medicine, Berlin

Impairments in follicle-stimulating hormone (FSH) synthesis or action have profound effects on mammalian fertility. Mechanisms controlling FSH synthesis by pituitary gonadotrope cells are incompletely understood. Previously, we identified a critical role for forkhead protein L2 (FOXL2) in activin-stimulated FSHβ subunit (*Fshb*) transcription *in vitro*. To examine the role of pituitary FOXL2 *in vivo*, we generated a gonadotrope-specific *Foxl2* knockout model (hereafter cKO). *Foxl2* cKO mice developed overtly normally but their ovarian and testicular weights were significantly reduced compared to controls. Subfertility was observed in both male and female cKO mice, resulting in smaller litter sizes. Ovaries of cKO females expressed FOXL2 at wild-type levels and responded normally to exogenous gonadotropins, ruling out an ovarian defect. In contrast, the numbers of naturally ovulated oocytes and corpora lutea counts were reduced in cKO relative to controls. cKO males exhibited abnormal testicular morphology and significantly reduced sperm counts. Serum FSH was dramatically reduced in cKO males and a decrease was also observed in females. To determine whether the observed FSH deficiency might derive from impaired activin signaling, we performed *ex vivo* recombination in primary pituitary cultures and observed attenuation in both basal and activin A-induced *Fshb* transcripts as well as secreted FSH, without affecting luteinizing hormone release. Collectively, these data provide the first definitive demonstration of a gonadotrope-restricted transcription factor required for the selective regulation of FSH production *in vivo*. In the absence of FOXL2, activin-stimulated *Fshb* expression and FSH secretion are reduced, leading to impairments in follicle development, spermatogenesis, and fertility.

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EXPRESSION OF DEADENYLASES IN GROWING MURINE OOCYTES

Karl-Frederic VIEUX [1,2,3], Joao SUZUKI [3], and Hugh CLARKE [1,2,3]
[1]Department of Obstetrics and Gynecology, McGill University, Montreal, Quebec, Canada
[2] Department of Biology, McGill University, Montreal, Quebec, Canada
[3] Research Institute, McGill University Health Center, Montreal, Quebec, Canada

During its growth, the oocyte stores large amounts of mRNA before downregulating transcription. These mRNA molecules are kept in a quiescent state until they are recruited in a temporally specific manner when needed. This crucial silencing has generally been thought to be induced by deadenylation of the 3'-poly(A) tail. Many deadenylases have been described and characterized, but none have been shown to be directly involved in deadenylation in mammalian oocytes. We set out to determine which deadenylases are involved in the process. Using RT-PCR, we show the expression of numerous deadenylases in murine oocytes, including CNOT6, a subunit of a major cytoplasmic deadenylase. To further characterize the expression of CNOT6, we stained oocytes using an anti-CNOT6 antibody. We observe a nuclear signal at early stages of oogenesis that disappears as the oocyte grows. In the cytoplasm, we detect immuno-reactive foci that accumulate while the cell grows, concentrate in the cortex and colocalize with the cortical actin cytoskeleton. To assess the specificity of this immunoreactivity, we induced a knock-down using siRNAs targeting Cnot6. RT-PCR confirmed depletion of Cnot6, and preliminary results indicate that cortical anti-CNOT6 staining was also reduced. Our results suggest that, in murine oocytes, CNOT6 may be expressed in the nucleus and in cytoplasmic particles early on in their development and that, as they grow, expression in the nucleus is lost while the immunoreactive cytoplasmic particles accumulate in the cortex.

CONFIRMATION OF PITX2C N-TERMINAL DOMAIN INTERACTING PROTEINS

Shian Yea Wong[1,3], Dora Siontas[2,3] & Aimee K. Ryan[1,2,3]
1. Department of Human Genetics, McGill University
2. Depeartment of Experimental Medicine, McGill University
3. RI MUHC

During embryogenesis, the patterning of the left-right axis is required to establish asymmetric organ morphogenesis. Pit2c, a bicoid-related homeodomain transcription factor, is one of the factors essential for this process. Pitx2c is asymmetrically expressed in the embryo on the left side of the lateral plate mesoderm and continues to be expressed on the left side of future asymmetric organs such as the heart and the gut. Previous work using loss-of-function and gain-of-function of Pitx2c showed that it is important in left-right patterning and that the N-terminal domain of Pitx2c is critical for interactions responsible for this function. To better understand the role of the Pitx2c N-terminus, we are identifying its protein interaction partners. Two yeast-two hybrid library screens using the chick Pitx2c N-terminus against a mouse adult cDNA library and the mouse Pitx2c N-terminus against a mouse E11 cDNA library were performed. Thirty-six candidate interaction partners were identified two or more times including proteins required for ubiquitination and translation. From the resulting proteins, we are confirming both their interaction with the Pitx2c N-terminus using a yeast two-hybrid switch vector test and their co-expression with the Pitx2c N-terminus by *in situ* hybridization and immunohistochemistry. Further functional analysis will be performed for candidates that meet both criteria.

BREAKTHROUGHS IN REPRODUCTION AND DEVELOPMENT

Research Day 2012

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

May 2, 2012

List of participants

Adel Reda Abouelmagd
Yassaman Aghazadeh**
Elie Akoury*
Wagdan Alenizy
Shihadeh Anani*
Asangla Ao
Raheleh Aram
Janice Bailey
EunJin Bang*
Serena Banh**
Amanda Baumholtz*
Nathalie Bédard
Richard Behringer***
Robert Berger**
Daniel Bernard
Erika Bezerra de Menezes*
Rodrigo Bohrer
Annie Boisvert
Patrick Boisvert
Elise Boivin-Ford
Rohini Bose*
Laura Burga
Enrico Campioli
Douglas Carrell***
Donovan Chan
Adrienne Chu
Hugh Clarke
Michelle Collins**
Martine Culty
Edward Daly
Caroline Dayan
Jurrien Dean***
Christine Dery
Anne Marie Downey*
Daniel Dufort
Julie Dufresne
Raj Duggavathi
Lisa Dupuis*
Nicole Edwards
Stephany El Hayek
Nazem El Husseini
Sheila Ernest
Charles Essagian
Maria Teresa Eyzaguirre
Jinjiang Fan*
Mena Farag
Omar Farah*
Sahmi Fatiha
Jerome Fortin
Justine Garner
Yasmine Ghorayeb
Nadiya Goswami
Hilda Morayma Guerrero-Netro
Barbara Hales
Taghreed Heba*
Belen Herrero
Dominic Hou*
Chunwei Huang
Leeyah Issop
Aliea Jamal
Loydie Jerome-Majewska***
Halim Khairallah
Dayana Krawchuk
Claudia Lalancette*
Romain Lambrot
Mylène Landry
Jiehan Li
Xinfang Li
Yining Li
Yiran Li
Trang Luu
Yi-Qian Ma
Marion Mandon
Puttaswamy Manjunath
Gurpreet Manku**
Josée Martel
Daniel Martinez*
Jennifer Maselli
Serge McGraw
Joana Mezzalira
Patricia Monnier
Maira Alejandra Moreno-Garcia*
Tania Morielli**
Makoto Nagano
Thomas Nardelli*
Ngoc Minh Phuong Nguyen
Cristian O'Flaherty
Lain Ohlweiler
Burak Ozkosem
Melissa Pansera
France-Hélène Paradis
Stephanie Park
Catriona Paul
Geneviève Plante*
Christopher Price
Ramesh Reddy*
Carlis Rejon
Mariana Remiao
Timothée Revil*
Ayman Rezk
Aimee Ryan
Monica Sakai
Mahutin-Vianney Salmon
Maria San Gabriel
Veronica Sanchez Gonzalez*
Sathvika Sanjiv Jagannathan
Oli Sarkar**
Ava Schlisser
Johanna Selvaratnam
Marian Shafik*
Dayananda Siddappa*
Keith Siklenka*
Annie Simard
Florian Storch***
Teruko Taketo
Stella Tran*
Jacquetta Trasler
Emilie-Tévy Troeung
Karl Vieux*
Yao Wang
Ying Wang
Simon Wing
Shian Yea Wong*
Bao Zeng Xu
Yojiro Yamanaka
Ahmet Yilmaz
Berthe Youness
Krista Zeidan
Jibin Zeng
Jacques Zhang
Li Zhang
Xiangfan Zhang
Xiang Zhou

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

NOUVELLES AVANCÉES EN REPRODUCTION ET DÉVELOPPEMENT

Journée de recherche 2012

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

le mercredi 2 mai 2012

Liste des participants

Adel Reda Abouelmagd
Yassaman Aghazadeh**
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Carlis Rejon
Mariana Remiao
Timothée Revil*
Ayman Rezk
Aimee Ryan
Monica Sakai
Mahutin-Vianney Salmon
Maria San Gabriel
Veronica Sanchez Gonzalez*
Sathvika Sanjiv Jagannathan
Oli Sarkar**
Ava Schlisser
Johanna Selvaratnam
Marian Shafik*
Dayananda Siddappa*
Keith Siklenka*
Annie Simard
Florian Storch***
Teruko Taketo
Stella Tran*
Jacquetta Trasler
Emilie-Tévy Troeung
Karl Vieux*
Yao Wang
Ying Wang
Simon Wing
Shian Yea Wong*
Bao Zeng Xu
Yojiro Yamanaka
Ahmet Yilmaz
Berthe Youness
Krista Zeidan
Jibin Zeng
Jacques Zhang
Li Zhang
Xiangfan Zhang
Xiang Zhou

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