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 1, 2013

La Plaza - Holiday Inn Montréal Midtown
420 Sherbrooke Ouest
Montréal, Québec

Centre universitaire de santé McGill
McGill University Health Centre

McGill

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

HUMAN REPRODUCTION AND DEVELOPMENT

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8:00 AM	Registration and coffee / Poster set-up
8:50	Opening remarks: **Dr. Martine Culty**
9:00	**Dr. Stephanie Seminara, Harvard Medical School, Reproductive Endocrine Unit at Massachusetts General Hospital,** *"The Hypothalamic Control Of Reproduction: A Kiss To Remember"* – introduced by Dr. Daniel Bernard
9:45-10:45	Oral presentations (Chairs: **Michelle Collins and Karl Vieux**) **O-01. Jérôme Fortin.** "*Gonadotrope Expression Of Smad4 Is Required For Normal FSH Synthesis And Fertility In Mice*" **O-02. Stephany El-Hayek.** "*Follicle-stimulating Hormone Regulates Contact And Communication Within The Mouse Ovarian Follicle*" **O-03. Dayananda Siddappa.** "*Transient Inhibition Of Mitogen-Activated Protein Kinase Activity In Preovulatory Follicles Abolishes The Ovulatory Response To Gonadotropins In Mice*" **O-04. Serge McGraw.** "*Sustained Loss Of DNA Methylation At Imprinted-Like Loci Following Transient DNMT1 Deficiency In Mouse ES Cells*"
10:45-11:05	Health Break
11:05-11:35	**Dr. Yojiro Yamanaka, Assistant Professor, Goodman Cancer Research Centre, Department of Human Genetics, McGill University,** *"Specification And Allocation Of The First Embryonic Lineages In The Mouse Blastocyst"* – introduced by Dr. Daniel Dufort
11:35-12:20	**Dr. Anna-Katerina Hadjantonakis, Sloan-Kettering Institute,** *"Guts And Gastrulation: Cell Dynamics And The Morphogenesis Of The Early Mouse Embryo"* – introduced by Dr. Loydie Jerome-Majewska
12:20-1:50	*Lunch / Poster Session* **P-01. Cécile Adam**, "*Activating Protein-2 (Ap2) And Specifity Protein-1 (Sp1) Are Implicated In The Transcriptional Regulation Of The Connexin26 Gene In The Epididymis*" **P-02. Yasaman Aghazadeh**, "*14-3-3ε Is An Outer Mitochondrial Membrane Scaffold Which Buffers The Role Of TSPO In Steroidogenesis In MA-10 Cell*

Line"
P-03. Océane Albert, "*Human Testis Steroidogenesis Is Inhibited By Phthalates*"
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P-20. Taghreed Heba, "*Uncovering The Function Of TMED2 During Trophoblast Differentiation*"
P-21. Louis Hermo "*Proteomics Characterization Of The Cytoplasmic Droplet Of Epididymal Sperm*"
P-22. Dominic Hou, "*Is TMED2 Essential In The Chorion For Normal Interaction Between The Allantois And The Chorion In Mice?*"
P-23. Leeyah Issop, "*Role Of ATAD3 In Inter-Organelle Interactions In Hormone-Induced Leydig Cell Steroidogenesis*"
P-24. Romain Lambrot, "*The Histone H3 Lysine 4 Demethylase, KDM1A Is Essential For Meiotic Progression And The Survival Of Spermatogonial Stem*

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P-25. Pavine Lefevre, "*Effects Of Brominated Flame Retardants On Human Granulosa Cells*"
P-26. Lundi Ly, "*Aberrant DNA Methylation Patterns In BALB/c Sperm As A Result Of MTHFR Deficiency In Combination With Or Without Folate Deficiency And Supplementation*"
P-27. Marion Mandon, "*Tricellulin Is Implicated In The Tight Junctions Of The Blood-Epididymis Barrier And Is Related To Basal Cell Projections*"
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P-38. Shian Yea Wong, "*Pitx2c N-terminus Candidate Interaction Partners Regulate Gene Expression*"

1:50-2:20 **Dr. Rima Slim, Assistant Professor, Human Genetics and Obstetrics/Gynecology, Montreal General Hospital Research Institute,** *"The Genetics Of Hydatidiform Moles: Recent Advances And Role Of NLRP7 In Mole Formation"* – introduced by Dr. Asangla Ao

2:20-3:05 Oral presentations (Chairs: **Serge McGraw and Gurpreet Manku**)
O-05. Adel Moawad. "*L-carnitine Supplementation During Vitrification Of Mouse Oocytes At The Germinal Vesicle Stage Improves Spindle Configuration And Subsequent Pre-Implantation Development Following IVM And IVF*"
O-06. Elsa Kichine. "*Proteomic Profiling Of The Sperm Heads From Infertile Men Reveals Decreased Expression Of Five Members Of The Chaperonin Containing TCP-1 Complex*"
O-07. Nazem El Husseini. "*Does Nitrosative Stress Play A Role In Mediating The Embryotoxicity Induced By Hydroxyurea?*"

3:05-3:25	Health Break
3:25-4:10	**Dr. Erika Matunis, Department of Cell Biology, John Hopkins University School of Medicine,** *"Stem Cell Renewal In The Drosophila Testis"* – introduced by Dr. Hugh Clarke
4:10-5:00	Oral presentations (Chairs: **Timothée Revil and Rohini Bose**) **O-08. Catriona Paul.** "*Aging Is Associated With Altered Gene Expression And A Reduced Number And Quality Of Spermatogonial Stem Cells*" **O-09. Shawn Fayer.** "*Dynamics Of Response To Asynapsis And Meiotic Silencing In Spermatocytes From Robertsonian Translocation Carriers*" **O-10. Keith Siklenka.** "*Overexpression Of The Histone H3 Demethylase KDM1A In Male Germ Cells Alters The Sperm Epigenome, Causes Abnormal Offspring Development And Is Implicated In Transgenerational Epigenetic Inheritance*"
5:00-5:15	Award Presentation and Concluding Remarks: **Dr. Hugh Clarke**
5: 15 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 2013

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

le mercredi 1 mai 2013
La Plaza - Holiday Inn Montréal Midtown, 420 Sherbrooke Ouest, Montréal, Québec

8 h — Inscription et café / Installation des affiches

8 h 50 — Mot de bienvenue : **Dr. Martine Culty**

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10 h 45 — Pause café

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13 h 50 — **Dr. Rima Slim, Assistant Professor, Human Genetics and Obstetrics/Gynecology, Montreal General Hospital Research Institute,** "*The Genetics Of Hydatidiform Moles: Recent Advances And Role Of NLRP7 In Mole Formation*" – presentée par Dr. Asangla Ao

14 h 20 — Présentations orales (Modérateurs : **Serge McGraw et Gurpreet Manku**)
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15 h 05 — Pause café

15 h 25 **Dr. Erika Matunis, Department of Cell Biology, John Hopkins University School of Medicine,** *"Stem Cell Renewal In The Drosophila Testis"* – presentée par Dr. Hugh Clarke

16 h 10 Présentations orales (Modérateurs : **Timothée Revil et Rohini Bose**)
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17 h Présentation de prix et mot de conclusion : **Dr. Hugh Clarke**

17 h 15 Démontage des affiches

Invited Speakers
Présentateur et présentatrices invités

Dr. Stephanie Seminara, Harvard Medical School, Reproductive Endocrine Unit at Massachusetts General Hospital (9:00 – 9:45 a.m.) "*The Hypothalamic Control Of Reproduction: A Kiss To Remember*"

Dr. Yojiro Yamanaka, Assistant Professor, Goodman Cancer Research Centre, Department of Human Genetics, McGill University (11:05 – 11:35 a.m.) "*Specification And Allocation Of The First Embryonic Lineages In The Mouse Blastocyst*"

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Dr. Rima Slim, Assistant Professor, Human Genetics and Obstetrics/Gynecology, Montreal General Hospital Research Institute (1:50 – 2:20 p.m.) "*The Genetics Of Hydatidiform Moles: Recent Advances And Role Of NLRP7 In Mole Formation*"

Dr. Erika Matunis, Department of Cell Biology, John Hopkins University School of Medicine (3:25 – 4:10 p.m.) "*Stem Cell Renewal In The Drosophila Testis*"

TITLE: THE HYPOTHALAMIC CONTROL OF REPRODUCTION: A KISS TO REMEMBER

Stephanie Seminara
Harvard Medical School, Reproductive Endocrine Unit at Massachusetts General Hospital Boston, Massachusetts USA

Education:

Harvard College, Cambridge, MA	B.A.	1987	Biology
Harvard Medical School, Boston, MA	M.D.	1991	Medicine

Positions Held:

Academic Appointments:

1997	Instructor in Medicine, Harvard Medical School	
2002	Assistant Professor of Medicine	Harvard Medical School
2008	Associate Professor of Medicine	Harvard Medical School

Hospital or Affiliated Institution Appointments:

1994 -1997	Clinical/Research Fellow in Endocrinology, Massachusetts General Hospital
1998 – 2004	Assistant in Medicine. Massachusetts General Hospital
2004	Assistant Physician, Massachusetts General Hospital
2007	Associate Member, Center for Human Genetics Research, Massachusetts General Hospital

Abstract:

The hypothalamic hormone GnRH has traditionally been viewed as a central driver of the hypothalamic-pituitary-gonadal axis. Pulsatile GnRH release is required for pulsatile gonadotropin secretion, which then modulates gonadal steroid feedback, and brings about full fertility in the adult. Pathways governing GnRH neuronal ontogeny and function have been discovered by studying humans with disorders of GnRH secretion. The human genetics of patients with diverse reproductive phenotypes will be explored.

SPECIFICATION AND ALLOCATION OF THE FIRST EMBRYONIC LINEAGES IN THE MOUSE BLASTOCYST

Yojiro Yamanaka, Shihadeh Anani, Nobuko Yamanaka and Dayana Krawchuk
Goodman Cancer Research Centre, Department of Human Genetics, McGill University, Montreal, Quebec H3A 1A3 Canada

Biosketch:
Dr. Yojiro Yamanaka is a mouse geneticist and developmental biologist. He did his postdoctoral training in the laboratory of Dr. Janet Rossant who is one of pioneers and leading scientists in the fields of mammalian development and stem cells. In the Rossant lab, he developed various unique techniques to analyze preimplantation and gastrula mouse embryos such as a method to microinject small molecules (mRNAs, cDNAs etc) into early mouse blastomeres and a live imaging system to observe developing pre- and post- implantation embryos. Since he started his own laboratory in 2009, his team has been focusing on molecular and cellular mechanisms of morphogenesis and cell lineage specification in the early mouse embryo. Currently, he is the director of the transgenic facility at the Goodman Cancer Research Centre. A part of his lab and the transgenic facility are working closely to improve and/or develop techniques to efficiently generate genetically modified mouse disease models, such as generating new ES lines from non-permissive mouse strains and developing new transgenic generation strategies using ZFNs/TALENs/CRISPR.

Education/Training

Mar. 1991	Hokkaido University, Japan	Bachelor	Pharmaceutical Science
Mar. 1993	Hokkaido University, Japan	M.Sc	Pharmaceutical Science
Mar. 1997	Osaka University, Japan	PhD	Medical Science
Apr. 1998	Osaka University, Japan	Postdoctoral	Medical Science
Dec. 2006	Hospital for Sick Children, Toronto	Postdoctoral	Developmental Biology

Positions

1997-1998	Postdoctoral fellow, Department of Molecular Oncology, Osaka University, Japan (Dr. Toshio Hirano)
1998-2000	Assistant Professor, Department of Molecular Oncology, Osaka University, Japan
2000-2005	Postdoctoral fellow, Samuel Lunenfeld Research Institute, Mt.Sinai Hospital, Toronto (Dr. Janet Rossant)
2005-2006	Postdoctoral fellow, Hospital for Sick Children, Toronto (Dr. Janet Rossant)
2007-2009	Research associate, Hospital for Sick Children, Toronto (Dr. Janet Rossant)
2009-	Assistant Professor, Goodman Cancer Research Centre, Dept. of Human Genetics, McGill University

Abstract:
At implantation, the mouse blastocyst consists of three lineages: epiblast (EPI), primitive endoderm (PE) and trophectoderm (TE). These three lineages are allocated in a specific configuration in the blastocyst: the TE forms the outer epithelial layer of the blastocyst, the PE forms the surface epithelial layer of the inner cell mass (ICM) and the EPI forms a packed cluster in the ICM. Although the formation of epithelia and the maintenance of epithelial integrity are

important for embryonic development, how these processes affect lineage specification and allocation in the blastocyst is still not fully known.

We are using mouse genetics, embryo manipulation and live imaging analysis to understand what controls lineage allocation and how it links with lineage specification in the blastocyst. During generation of the first two lineages, TE and ICM (identified as inner/outer cells after the 8-cell stage), we find that the acquisition of apico-basal cell polarity plays important roles for cell allocation and lineage specification. Apolar cells generated after the asymmetric division of 8-cell blastomeres, have an intrinsic ability to take an inner position and show the molecular characteristics of inner cells even before moving inward. Interestingly, if we block apical domain formation (thus blocking epithelium formation), outer cells show the molecular characteristics of inner cells. These results suggest that cells in the embryo use apico-basal polarity as a driver to allocate cells in the embryo as well as to link their positions with lineage specification. After cells are committed to one of the three lineages, epithelial integrity is required for maintaining lineage configuration in the blastocyst during blastocyst expansion. We find that removing Lkb1 (a tumor suppressor and modulator of cell polarity and epithelial integrity) from the embryo, causes defects in the positional configuration of the three lineages. Taken together, establishing apico-basal polarity to form epithelia and maintaining epithelial integrity play important roles for specification and allocation of the first embryonic lineages in the mouse blastocyst.

GUTS AND GASTRULATION: CELL DYNAMICS AND THE MORPHOGENESIS OF THE EARLY MOUSE
EMBRYO

Anna-Katerina Hadjantonakis
Developmental Biology Program, Sloan-Kettering Institute, New York, NY 10065, USA

Biosketch:

My research interests center on understanding cell lineage development, tissue patterning and morphogenesis. Towards this goal my laboratory is investigating the cellular and molecular mechanisms underlying the growth, differentiation and patterning of early mammalian embryos using the mouse as a model system. We focus on two key developmental stages when the events of lineage commitment, patterning and morphogenesis converge, these being the formation of the pre-implantation blastocyst and the post-implantation gastrula.

My laboratory exploits cutting-edge methods to address critical questions pertaining these critical developmental events. In doing so we have made pioneering and contributions to our understanding of blastocyst development (the focus of this application), in addition to redefining our understanding of gastrulation, in terms of cell fate, cell behaviors and morphogenetic mechanisms (the subjects of our other funded projects).

Throughout my career live imaging has been a hallmark of my approach to biological research. I strongly believe that live imaging provides an unprecedented level of resolution, and represents an essential methodology for understanding the dynamics of living systems. Towards this aim my laboratory has pioneered the development and optimization of optical probes, reporter expressing strains and methods for live imaging cells in developing mouse embryos and embryo-derived stem cells.

Education/Training

Imperial College, University of London, UK	BSc	1986-1990	Biochemistry
Imperial College, University of London, UK	PhD	1990-1995	Molecular Genetics
Samuel Lunenfeld Research Institute, Toronto, Canada		1996-2000	Mouse Embryology
Columbia University, New York, USA		2000-2003	Mouse Embryology

Positions

2009 -	Associate Member, Sloan-Kettering Institute, New York
2004 - 2009	Assistant Member, Sloan-Kettering Institute, New York
2000 - 2003	Postdoctoral Fellow, Columbia University, New York
1996 - 2000	Postdoctoral Fellow, Samuel Lunenfeld Research Institute, Toronto

Abstract:

Gastrulation is a paradigm for tissue morphogenesis, as it involves the coordination of cell fate specification and cell movement, which together drive tissue formation and segregation. In the mouse, gastrulation transforms a cup-shaped structure comprising two tissue layers (epiblast and visceral endoderm) and separated by a single basement membrane, into one comprising three tissue layers (epiblast, mesoderm and gut endoderm) and two basement membranes. Our studies are focused on understanding the coordinate events driving this transformation.
Live imaging combined with genetic labeling studies suggest that morphogenesis

of gut endoderm in the mouse embryo involves a dynamic widespread intercalation between two endodermal populations, definitive and visceral. This morphogenetic event results in the formation of an epithelium on the surface of the embryo, comprising cells of two distinct origins. By investigating mutants in which gut endoderm morphogenesis is perturbed we are developing a mechanistic understanding of the cell behaviors regulating this process. Definitive endoderm cells adopt a trajectory aligned with the wings of mesoderm as they exit the primitive streak. From there they emerge on the surface of the embryo, by egressing into the visceral endoderm epithelium. In doing so they execute a program of mesenchymal-to-epithelial transformation (MET) involving the acquisition of apico-basal polarity and resulting in the co-ordinate assembly of a basement membrane at the gut endoderm / mesoderm interface, a fundamental feature of gastrulation. Progress with these studies will be discussed.

THE GENETICS OF HYDATIDIFORM MOLES: RECENT ADVANCES AND ROLE OF NLRP7 IN MOLE FORMATION

Rima Slim

Departments of Human Genetics and Obstetrics and Gynecology, McGill University Health Centre, Montreal H3G 1A4, Canada.

Biosketch:

Trained in Human Genetics at the Pasteur Institute in Paris, France where she performed her Ph.D. and postdoctoral studies. She established her first independent research group at the American University of Beirut, Lebanon in 1994 where she initiated research work on the genetics of familial recurrent hydatidiform moles.

In 2002, she moved to McGill University where she implemented her research work and is now Associate Professor.

By studying rare familial cases of recurrent hydatidiform moles, her group identified the first and major maternal-effect gene, NLRP7, for this condition. Her research work is currently focused on (i) the understanding of the functional role of NLRP7 in the pathology of moles, (ii) determining its involvement in other forms of reproductive loss, and (iii) identifying new genes for reproductive wastage, an understudied area of medicine affecting more than 20% of clinically recognized pregnancies.

Abstract:

Hydatidiform mole is a human pregnancy with no embryo. The paradoxical nature of this aberrant pregnancy has fascinated and puzzled scientists in all civilizations. Common HMs occur once in every 600 pregnancies in western countries but have 2 to 10 times higher frequencies in Asian countries. Common moles are sporadic, not recurrent, and have complex multifactorial etiologies. By studying rare familial forms of recurrent hydatidiform moles (RHMs), two maternal-effect genes responsible for this condition have been identified. NLRP7 codes for a nucleotide oligomerization domain-like receptor protein and is mutated in 60 to 80% of RHMs cases. KHDC3L codes for a KH domain containing 3-like, subcortical maternal complex member, and is mutated in 5 to 14% of NLRP7-negative patients. Patients with two mutations in any of these two genes usually fail to have normal pregnancies. The exact molecular functions of these two genes and how they lead to moles is not fully understood. Data from various groups indicate that mutations in these two genes do not affect ovulation, fertilization, but abnormalities in the embryos of the patients manifest after the formation of the zygote and/or during early development. These observations are in line with the emerging views about the mechanisms leading to all types of moles and are corroborated by reports of successful pregnancies after oocyte donation in patients with two NLRP7 mutations. My talk will be centered around our current understanding of the functional roles of NLRP7 in early human development and the mechanisms of mole formation.

STEM CELL RENEWAL IN THE DROSOPHILA TESTIS.

Phylis Hétié, Margaret De Cuevas, Erika Matunis
Department of Cell Biology, John Hopkins University School of Medicine, Baltimore, Maryland USA

Biosketch:
I have been studying stem cells in the Drosophila testis since I began my post-doctoral fellowship with Steve DiNardo, at Rockefeller over twenty years ago. My lab has made important contributions to the understanding of stem cell biology by developing the Drosophila testis as a model stem cell system. When we began, stem cells had been characterized in the fly ovary, but not in the testis. The ability to apply genetics to identify and characterize the stem cells in an intact tissue led us to provide one of the first descriptions of a stem cell niche at the cellular and molecular level in vivo. Upon establishing my own lab at the Carnegie Institution, we found that Jak-Stat signaling is a critical component that molecularly defines this niche. Subsequently, we discovered that spermatogonia can dedifferentiate to replace lost germline stem cells, and that the two stem cell lineages that sustain spermatogenesis (germline and somatic) compete for space within the niche via regulated adhesion. We continue to use the fly testis to address questions of broad significance we hope will inform our understanding of stem cell research more generally; others have confirmed that spermatogonial dedifferentiation occurs in mammalian testes, and we have recently moved on to uncover epigenetic and hormonal signals in our model system. I am fortunate to present our work to diverse audiences around the world, and I appreciate the challenge of being immersed in several fields including stem cell biology developmental biology, reproductive biology, cell biology, and genetics.

Education/Training

1986	B.S.	Pacific Lutheran University	Biology
1992	Ph.D.	Northwestern University	Cell Biology
1998	Postdoctoral	The Rockefeller University	Genetics

Positions

1983 - 1986 Undergraduate Researcher
Advisor: Dr. Michele Crayton Pacific Lutheran University Tacoma, WA

1986 - 1990 Graduate Research Fellow
Mentor: Dr. Gideon Dreyfuss Northwestern University Chicago, IL

1990 - 1992 Graduate Research Fellow
Mentor: Dr. Gideon Dreyfuss University of Pennsylvania Philadelphia, PA

1992 - 1993 Postdoctoral Associate
Mentor: Dr. Gideon Dreyfuss University of Pennsylvania Philadelphia, PA

1993 - 1998 Postdoctoral Fellow
Mentor: Dr. Stephen DiNardo Rockefeller University New York, NY

1998 - 2002 Staff Associate,
Department of Embryology Carnegie Institution Baltimore, MD

2002 - 2008 Assistant Professor,
Department of Cell Biology Johns Hopkins U. School of Medicine Baltimore, MD

2008 - present Associate Professor,
Department of Cell Biology Johns Hopkins U. School of Medicine Baltimore, MD

Abstract:
Stem cells often reside in specific local microenvironments, or niches, where signals from nearby cells and substrates promote stem cell maintenance. Spermatogonial stem cells in the testis of both vertebrates and invertebrates provide a lifetime supply of sperm, making this tissue an excellent model for studying stem cell biology. We have focused on the Drosophila testis, as it contains a morphologically distinct niche that can be probed with sophisticated genetic tools. The apex of each Drosophila testis contains a single niche comprised of a cluster of quiescent somatic cells called the hub, to which spermatogonial stem cells and somatic cyst stem cells (CySCs) adhere. We previously found that genetic ablation of spermatogonial stem cells prompts their differentiating progeny (called spermatogonia) to enter the niche and revert to functional spermatogonial stem cells. This was subsequently shown to occur in the mouse testis, underscoring its generality. Here, by genetically ablating CySCs, we find that hub cells are not only quiescent niche cells: they are also a reserve population of CySCs. Ablation of CySCs prompts hub cells to undergo an epithelial-to-mesenchymal transition via downregulation of Ecadherin. Delamination of cells from the hub is accompanied by a loss of hub cell quiescence. Hub cell proliferation is transient, however, and ceases when the niche is repopulated. The transdifferentiation of hub cells to CySCs requires the ablation of most or all CySCs, suggesting that CySCs normally actively signal to hub cells to maintain hub cell quiescence. Consistent with this hypothesis, hub cell transdifferentiation does not replenish CySCs that are lost during normal aging. Our work suggests that the ability of quiescent cells to transdifferentiate into stem cells in response to stem cell depletion may be a previously undiscovered feature of niches in general.

Oral Presentations
Présentations orales

1. Jérôme Fortin (9:45 a.m.)
2. Stephany El-Hayek (10:00 a.m.)
3. Dayananda Siddappa (10:15 a.m.)
4. Serge McGraw (10:30 a.m.)

5. Adel Moawad (2:20 p.m.)
6. Elsa Kichine (2:35 p.m.)
7. Nazem El Husseini (2:50 p.m.)

8. Catriona Paul (4:10 p.m.)
9. Shawn Fayer (4:25 p.m.)
10. Keith Siklenka (4:40 p.m.)

HORMONE-INDUCED 14-3-3Γ ADAPTOR PROTEIN REGULATES STEROIDOGENIC GONADOTROPE EXPRESSION OF SMAD4 IS REQUIRED FOR NORMAL FSH SYNTHESIS AND FERTILITY IN MICE.

Jérôme Fortin[1], Chu-Xia Deng[2], Ulrich Boehm[3] and Daniel J Bernard[1]

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[2] NIDDK, National Institutes of Health, Bethesda, MD, United States
[3] Department of Pharmacology and Toxicology, University of Saarland School of Medicine, Homburg, Germany

Activins are potent regulators of follicle-stimulating hormone (FSH) synthesis by pituitary gonadotrope cells. FSH, in turn, acts on the gonads to stimulate steroidogenesis and gametogenesis, thus ensuring proper reproductive function and fertility. In vitro data suggest that activins bind to complexes of type I and type II serine/threonine kinase receptors at the surface of gonadotropes and thereby activate a signaling cascade that culminates in the transcription of the FSH β subunit gene (Fshb). This cascade involves activation of the receptor-regulated SMADs, SMAD2/3, which partner with SMAD4, accumulate in the nucleus, and directly bind to the Fshb promoter along with interacting transcription factors such as forkhead box L2 (FOXL2). However, it remains largely unclear whether this model applies in vivo. Unexpectedly, we recently observed that mice lacking the full-length forms of Smad2/3 in gonadotropes have normal FSH synthesis and fertility. To clarify the role of SMAD-dependent signaling in FSH synthesis, we ablated Smad4 selectively in gonadotropes using a Cre/lox strategy (hereafter S4cKO mice). S4cKO mice showed hypogonadism: males had small testes and impaired sperm production, whereas females were subfertile secondary to impaired ovarian follicle maturation beyond the pre-antral stage. These phenotypes were likely explained by FSH deficiency, as circulating FSH levels and pituitary Fshb expression were decreased in both males and females S4cKO mice compared with control littermates. When cultured ex vivo, primary pituitary cells from S4cKO mice had much lower basal and activin A-stimulated Fshb expression compared to pituitary cells from control littermates. Collectively, these results reveal a necessary role for SMAD signaling in gonadotropes to maintain proper FSH synthesis in vivo.

This work was supported by a Graduate Scholarship from CIHR to JF, and operating grant CIHR MOP-89991 to DJB.

FOLLICLE-STIMULATING HORMONE REGULATES CONTACT AND COMMUNICATION WITHIN THE MOUSE OVARIAN FOLLICLE

Stephany EL-HAYEK [1,2,3] and Hugh CLARKE [1,2,3]

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[2] Department of Biology, McGill University, Montreal, Quebec, Canada
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Throughout folliculogenesis, the ovarian follicle functions as a single physiological unit:its different compartments maintain contact and communication, which is essential for development of both somatic and germ components. Also crucial for folliculogenesis is follicle-stimulating hormone (FSH), whose role during its late stages is well-established. It remains unclear however, how FSH affects the oocyte's developmental competence, acquired during late folliculogenesis. We previously showed that, embryos derived from oocytes of mice carrying a targeted mutation in the Fshb gene arrest at early cleavage. To identify the origin of the defect in the Fshb-/- oocytes, we examined cellular interactions within the follicle. We observed that fewer granulosa cells surrounded oocytes of Fshb-/- mice than those of Fshb+/- mice. We then examined expression of genes encoding key cell-adhesion and gap-junction components. We observed that, in Fshb+/- females during the FSH-dependent transition from secondary to antral follicle, the quantity of Gja1 (connexin-43) and Cdh2 (N-cadherin) increased granulosa cells, and Gja4 (connexin-37) and Cdh1 (E-cadherin) increased in oocytes. In Fshb-/- follicles, this increase was absent or significantly reduced. We then compared expression levels in age-matched Fshb+/- and Fshb-/- follicles. We observed lower levels of all four mRNAs and of connexin-43 and N-cadherin protein in the mutants. We next assessed the density of trans-zonal projections (TZPs), cytoplasmic extensions of the granulosa cells that make contact with the oocyte. The density of total and actin-based TZPs was shown to be significantly higher and lower, respectively, in Fshb-/- oocytes. We conclude that FSH maintains normal expression of junctional gene products and proper distribution of TZPs.

TRANSIENT INHIBITION OF MITOGEN-ACTIVATED PROTEIN KINASE ACTIVITY IN PREOVULATORY FOLLICLES ABOLISHES THE OVULATORY RESPONSE TO GONADOTROPINS IN MICE

Dayanada Siddappa[1], Élaine Beaulieu[2], Nicolas Gévry[2], Philippe Roux[3] and Raj Duggavathi[1]

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[3] Institute for Research in Immunology and Cancer, Université de Montréal, Montreal, QC H3C 3J7.

Luteinizing hormone (LH) induces dramatic gene expression in granulosa cells of the preovulatory follicle. There is evidence that the mitogen-activated protein kinase 3/1 (Mapk3/1) pathway plays a critical role during this LH signaling. However, the precise mechanisms remain incompletely understood. We hypothesized that Mapk pathway regulates ovulation through transcriptional regulation of ovulatory genes. To test this, LH-induced Mapk3/1 activity was inhibited using PD0325901, an inhibitor of mitogen-activated protein kinase-kinase. Immature (~23 d) mice received either PD0325901 (25 µg/g, i.p.; PD group) or vehicle (CON group) at 2 h before hCG stimulation during superovulation. MEK-inhibitor treatment abolished hCG-induced Mapk3/1 phosphorylation in granulosa cells at 1 h and 4 h post-hCG. While CON mice ovulated normally, there were no ovulations in PD mice. Histology of PD ovaries showed trapped oocytes that were surrounded by compact cumulus cells. By qPCR, there was expected hCG-driven increase in mRNA abundance of *Pgr, Ptgs2, Tnfaip6, Adamts1, Has2, Areg* and *Ereg* in CON granulosa cells, which was reduced ($P < 0.05$) in PD group. Downregulation of these ovulatory genes was associated with reduced mRNA ($P < 0.001$) and protein levels of the transcription factor, early growth response 1 (Egr1) in granulosa cells of PD mice. To link reduced Mapk3/1 activity to the downregulation of Egr1, we explored the phosphorylation status of the serum response factor (Srf), a well-characterized substrate of Mapk3/1. Abundance of phosphorylated isoform of Srf was lower in PD granulosa cells indicating that post-translational modification of Srf was affected by PD treatment. We then used a mouse granulosa cell line (GRMO2 cells) to test if Egr1 is recruited to promoter regions of the *Ptgs2* and *Adamts1* genes, by chromatin immunoprecipitation and qPCR (ChIP-qPCR). GRMO2 cells were treated with cAMP for 4h to induce hCG-driven genes and harvested for ChIP with either Egr1 antibody or normal rabbit IgG. Enrichment of the promoter regions containing Egr1 binding-sites in immunoprecipitants of Egr1 antibody indicated that Egr1 interacts with *Ptgs2* and *Adamts1* promoters. These data demonstrate that a transient inhibition of MAPK signaling during early hours of hCG stimulation is sufficient to abrogate ovulation in mice. Thus, we conclude that Mapk3/1 regulates ovulation, at least in part, through Srf, thereby regulating the transcription of *Egr1* and its target genes, *Ptgs2* and *Adamts1* in granulosa cells of the preovulatory follicles.

SUSTAINED LOSS OF DNA METHYLATION AT IMPRINTED-LIKE LOCI FOLLOWING TRANSIENT DNMT1 DEFICIENCY IN MOUSE ES CELLS

Serge McGraw[1], Jacques X. Zhang[1], Mena Farag[1], Carolin Konermann[2], Maxime Caron[4], Christopher C. Oakes[2], K. Naga Mohan[3], Christoph Plass[2], Tomi Pastinen[4], Guillaume Bourque[4], J. Richard Chaillet[3], Jacquetta M. Trasler[1]

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[2]Epigenomics and Cancer Risk Factors Division, German Cancer Research Center, Heidelberg, Germany.
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Through every cycle of cell division, the epigenetic profile has to be accurately perpetuated in each of the daughter cells to preserve cell identity and fate. An important phase of maintenance of methylation patterns, initially established in a sex-specific manner (e.g. imprinted genes) in male and female germ cells, occurs during preimplantation development. However, the full extent of sequences that inherit methylation from sperm or the egg and must maintain these patterns in the early embryo is unknown. To investigate the consequences of perturbing the expression of the primary enzyme responsible for the maintenance of DNA methylation patterns, we generated a mouse ES-cell model with inducible repression of *Dnmt1*. Following transient (6 days) inactivation of *Dnmt1*, a significant reduction in genomic methylation was observed. Genome wide analysis by reduced representation bisulfite sequencing (RRBS) revealed that most loci regained their original methylation levels following the reactivation of *Dnmt1*, however many loci still had erroneous levels (hypo- and hypermethylation). Of particular interest is the "permanent" loss of DNA methylation at loci other than the known imprinted DMDs. DNA methylation is lost at these imprinted-like regions following inactivation of *Dnmt1* and is not regained upon its reactivation. Globally our results suggest that a failure to maintain methylation as a result of transient DNMT1 down-regulation in mouse ES cells is a model for the transient genomic methylation changes observed in early mammalian embryogenesis. Those sequences requiring continuous DNMT1 activity for their methylation maintenance extend beyond DMDs, and include sequences associated with normal development and sequences whose methylation patterns are altered in cancer.

L-CARNITINE SUPPLEMENTATION DURING VITRIFICATION OF MOUSE OOCYTES AT THE GERMINAL VESICLE STAGE IMPROVES SPINDLE CONFIGURATION AND SUBSEQUENT PRE-IMPLANTATION DEVELOPMENT FOLLOWING IVM AND IVF

Adel R. Moawad[1,5], Seang Lin Tan[1,4], Baozeng Xu[2], Hai Ying Chen[4], and Teruko Taketo[1,2,3]

Departments of Obstetrics and Gynaecology[1], Surgery[2] and Biology[3], McGill University, Montreal, Quebec. Montreal Reproduction Centre[4], Montreal, Quebec. Department of Theriogenology[5], Faculty of Veterinary Medicine, Cairo University, Giza, Egypt.

Oocyte cryopreservation is imperative for assisted reproductive technologies (ART). Although cryopreservation of metaphase II (MII) oocytes has been successfully reported, MII oocytes are vulnerable to the damage inflicted by the freezing procedure. Cryopreservation of germinal vesicle (GV) stage oocytes is an alternative option; however, blastocyst development from cryopreserved GV-oocytes is limited largely due to the requirement of in vitro maturation (IVM). We evaluated the effects of L-carnitine (LC) supplementation to the media during vitrification and thawing of mouse GV-oocytes and IVM on the pre-implantation development after IVF. LC is known to play an essential role in fat metabolism. Our results demonstrated that LC (0.6 mg/ml) supplementation to the IVM medium alone did not change the percentage of blastocyst development. However, LC supplementation to both vitrification and IVM media significantly improved blastocyst development to the levels comparable with those obtained from vitrified/thawed ovulated oocytes of both B6 and (B6.DBA)F1 mouse strains. To test the mechanisms by which LC induces its beneficial effects on embryonic development of vitrified/thawed GV-oocytes, we examined spindle organization and chromosome alignment in the MII-oocytes after IVM. We found that, the percentage of oocytes with normal spindle and chromosome alignment significantly decreased in vitrified group compared to the control without vitrification in the B6 strain; however the same conditions did not show a significant difference in the (B6.DBA)F1 strain. Supplementation of both vitrification and IVM media with LC significantly improved the percentage of oocytes with normal spindle and chromosome alignment to comparable levels as the control group without vitrification in the B6 strain. We are currently performing the same experiments in the (B6.DBA)F1 strain. Together, these results suggest that LC supplementation during vitrification and thawing of GV-oocytes improves spindle morphology and subsequent pre-implantation development. This novel approach of LC supplementation during vitrification could offer a positive impact on the efficiency of oocyte cryopreservation technology.

PROTEOMIC PROFILING OF THE SPERM HEADS FROM INFERTILE MEN REVEALS DECREASED EXPRESSION OF FIVE MEMBERS OF THE CHAPERONIN CONTAINING TCP-1 COMPLEX

Elsa Kichine[†], Barbara F. Hales[†], Bernard Robaire[†, §], and Peter Chan[‡]

[†]Department of Pharmacology and Therapeutics, McGill University, [§]Department of Obstetrics and Gynecology, [‡]Department of Urology, McGill University Health Centre, Montreal, Quebec, Canada.

The fertilization ability of spermatozoa depends on many biological processes, including the ability to undergo the acrosome reaction and capacitation. Since spermatozoa are transcriptionally silent, these processes depend on the post-translational modification of proteins and surface remodeling events that modify the sperm head surface architecture. The objective of this study was to use a proteomic approach to compare the sperm head protein profile of fertile and infertile men with idiopathic infertility (n=6/group) to identify potential biomarkers for male fertility. Three sperm samples obtained at three different time points, at 6-8 weeks apart,were provided by each subject for a total of 36 samples from the 12 men. Proteomic analysis of the sperm head fraction resulted in detection of 567 proteins. After the application of a cut-off, established by technical triplicate analysis for consistency of each sample, 124 proteins remained. The expression levels of the detected proteins were compared between groups using spectral counting methods. Interestingly, the expression of several families of proteins was decreased in the infertile men; these included proteasome subunits such as proteasome subunit alpha type-7 (3.7 fold), acrosome proteins such as acrosome binding protein (4.7 fold), elongation factors, and calcium transduction proteins. Most strikingly, the expression of five members of the chaperonin containing TCP-1 complex was markedly decreased; this included TCP-1 subunit gamma CCT3 (4.7 fold). To our knowledge, this is the first study that links the decreased expression of TCP-1 complex subunits with infertility in men. These findings give new insight into proteins that may regulate human fertility and identify potential protein targets for use in enriching spermatozoa that have greater fertilizing potential in samples from infertile men before IVF/ICSI cycles. Supported by the Canadian Institutes of Health Research.

DOES NITROSATIVE STRESS PLAY A ROLE IN MEDIATING THE EMBRYOTOXICITY INDUCED BY HYDROXYUREA?

Nazem El Husseini[1], Barbara Hales[1]

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Exposure to teratogenic doses of hydroxyurea triggers oxidative stress in embryos, leading to the modification of critical proteins. HU is metabolized to nitric oxide (NO). Although reactive nitrogen species (RNS) modify critical proteins, the role of RNS in mediating the embryotoxicity of HU has not been investigated. Our goal is to determine whether nitrosative stress plays a role in HU teratogenicity. Timed pregnant CD1 mice were treated with vehicle or HU (600 mg/kg) intraperitoneally on gestation day 9. Embryos were excised 0.5, 3 and 6 h post-treatment. NO concentrations were determined using a nitrate colorimetric assay (Enzo Life Sciences). Ss a measure of RNS activity, 3-nitrotyrosine formation in embryos was examined with Western blot analysis and localized in embryos with immunofluorescence. Nitrate concentrations were not significantly affected by HU (c, 0.5h: 173.5 ± 19.2 µM [mean ±SEM], 3h: 201.2 ± 30.2 µM, 6h: 300 ± 74.4 µM; HU, 0.5h: 230.7 ± 48.8 µM, 3h: 267.7 ± 49.3 µM, 6h: 224.3 ± 22.9 µM; N=4-5, $p>0.05$). Western blot analysis revealed 3-nitrotyrosine labeled proteins in the ~95, 70 and 30 KDa regions but without significant differences in intensity between control and HU samples (0.5h: 1.17 ± 0.2 fold change, 3h: 1.23 ± 0.13; N=4-5, $p>0.05$). 3-nitrotyrosine immunofluorescence at 3h post-treatment was widespread but did not differ between control and treated embryos. Thus, the exposure of organogenesis stage embryos to HU did not increase nitrate concentrations or 3-nitrotyrosine formation, suggesting that HU does not induce nitrosative stress in murine embryos. Funded by CIHR.

AGING IS ASSOCIATED WITH ALTERED GENE EXPRESSION AND A REDUCED NUMBER AND QUALITY OF SPERMATOGONIAL STEM CELLS

C. Paul, M. Nagano and B. Robaire

Several studies have demonstrated a decline in the male reproductive system, sperm quality and thus fertility with advancing paternal age. Some of the main characteristics of 'testis aging' are testicular atrophy and an increase in oxidative stress and DNA damage. Whether the problems seen in spermatogenesis arise from the progenitor spermatogonial stem cells (SSCs), e.g., from an accumulation of DNA damage and/or mutations, or from the somatic niche, or indeed both is unclear. We hypothesize that with the continual divisions of SSCs that is necessary to maintain the germ cell population there is deterioration of these cells over time. To test this hypothesis we utilized eGFP transgenic Sprague Dawley rats, at 4 (young), 18 and 21 months (aged), that express GFP in germ cells only. These GFP-positive germ cells were used for transplantation into busulfan-treated nude mice. After 3 months the testes were removed and the number of colonies counted (represents the number of SSCs) and their length measured (represents the quality of the SSC). Our results show that aged rats (18 and 21 months) demonstrate lower numbers (less than 50%) of stem cells, than do young rats. In addition, the 21 month old rats produce significantly shorter colonies suggesting that with aging the number and quality of SSCs deteriorate. To determine the molecular changes occurring in SSCs with age we isolated CD9 positive cells using FACS and extracted RNA for a whole genome microarray. A number of changes in gene expression in the aged CD9 positive cells were noted including an upregulation of approximately 40 genes and a downregulation of over 400 genes in the aged compared to the young; an altered gene expression was found for many genes involved in mitosis and in DNA damage. These results suggest that whether or not the Sertoli cells change with age it is evident that the SSCs change at the molecular level to exert effects on their number and quality and thus may be one of the underlying causes of reduced reproductive outcome with advancing age. This work was supported by a grant from CIHR.

DYNAMICS OF RESPONSE TO ASYNAPSIS AND MEIOTIC SILENCING IN SPERMATOCYTES FROM ROBERTSONIAN TRANSLOCATION CARRIERS

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Failure of homologous synapsis during meiotic prophase triggers transcriptional repression, while asynapsis of autosomes resulting from autosomal translocations shows high variation in outcomes ranging from meiotic arrest to normal spermatogenesis. Such a variation may result from a less robust response to autosomal asynapsis; variable proportion of spermatocytes with meiotic silencing of unsynapsed autosomal regions; and/or the difference in functions of affected autosomal genes. To establish the dynamics of autosomal asynapsis and the proportion of spermatocytes with meiotic silencing of unsynapsed autosomes, we examined the localization of several markers of unsynapsed chromatin in the spermatocytes of Robertsonian translocation carrier mice. The localization of γH2Ax and H3.3S31 at unsynapsed autosomes is differed from that observed in the XY body. We also looked at the localization of H3K9AC, a mark of transcriptional activation, and RNA polymerase II to further assess the transcriptional state of unsynapsed autosomes. Histone variant H3.3S31, a mark of unsynapsed chromatin, was enriched at autosomal regions in 12% of metaphase I/anaphase I spermatocytes of Robertsonian translocation carriers compared to the 100% of nuclei with H3.3S31 enrichment at sex chromosomes. Our data suggests that stable meiotic silencing of unsynapsed autosomal regions occurs in a small proportion of spermatocytes of Robertsonian translocation carriers. We therefore conclude that meiotic silencing of the X and Y in spermatocytes may have evolved to ensure stability of silencing and proper chromosomal segregation of the sex chromosomes, whereas autosomal asynapsis is an error-prone process with a less predictable outcome.

OVEREXPRESSION OF THE HISTONE H3 DEMETHYLASE KDM1A IN MALE GERM CELLS ALTERS THE SPERM EPIGENOME, CAUSES ABNORMAL OFFSPRING DEVELOPMENT AND IS IMPLICATED IN TRANSGENERATIONAL EPIGENETIC INHERITANCE

Keith Siklenka[1], Serap Erkek[5], Maren Godmann[2], Romain Lambrot[2], Christine Lafleur[2], George Chountalos[1], Tamara Cohen[2], Marilene Paquet[4], Matthew Suderman[3], Mike Hallett[3], Antoine Peters[5] and Sarah Kimmins[1,2]

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[2] Department of Animal Science, McGill University, Montreal, Canada.
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[4] Comparative Medicine & Animal Resources Centre, McGill University, Montreal, Canada
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Increasing evidence suggests that the paternal environment can influence the health and development of future generations through a process called epigenetic inheritance. In order to determine the role of the sperm epigenome in offspring development, as well as the transmission of epigenetic mutations across generations, we designed a transgenic mouse model with an altered sperm epigenome. Transgenic mice specifically overexpress lysine specific demethylase 1 (KDM1A) in male germ cells. KDM1A removes di-methylation from histone H3 lysine 4 (H3K4m2), which has previously been localized to genes in sperm implicated in spermatogenesis and embryogenesis (Brykczynska et al, 2010; Hammoud et al 2009). Characterization of offspring sired by heterozygous KDM1A transgenics revealed reduced survivability and a range of developmental defects. Importantly, descendants of transgenics that did not carry the transgene themselves also sired abnormal offspring indicating they had inherited epigenomic regions in germ cell that were resistant to reprogramming. The offspring of non-transgenic descendants were examined over three generations. By the third generation, males who had a transgenic great-grandfather no longer sired abnormal offspring suggesting inherited epimutations were reset. Analysis of the sperm epigenome of transgenic mice by chromatin immunoprecipitation followed by genome wide sequencing (ChIP-Seq) revealed specific reductions of H3K4me2 at transcriptional start sites of over 2000 genes. Many of these genes are implicated in embryo development. Candidate genes were selected for DNA methylation analysis based on large losses of H3K4m2 in order to determine if DNA methylation was also disrupted in the sperm of transgenics and non-transgenic descendants. No significant differences in DNA methylation were detected indicating that altered sperm histone methylation is the major factor contributing to abnormal phenotypes of transgenic sired offspring.

Poster Presentations
Présentations par affiches

1. Cécile Adam
2. Yasaman Aghzadeh
3. Océane Albert
4. Wejdan Alenezi
5. Shihadeh Anani
6. Raheleh Aram
7. Amanda Baumholtz
8. Rodrigo Bohrer
9. Patrick Boisvert
10. Rohini Bose
11. Elliot Byrne
12. Flavia Lorena Carvelli
13. Michelle Collins
14. Nicole Edwards
15. Samar Elzein
16. Maria Teresa Eyzaguirre
17. Omar Farah
18. Marie-Lyne Fillion
19. Maira Alejandra Moreno Garcia
20. Taghreed Heba
21. Louis Hermo
22. Dominic Hou
23. Leeyah Issop
24. Romain Lambrot
25. Pavine Lefevre
26. Lundi Ly
27. Marion Mandon
28. Gurpreet Manku

29. Joana Mezzalira
30. Lain Ohlweiler
31. Burak Özkösem
32. Melissa Pansera
33. Timothée Revil
34. Monica Sakai
35. Johanna Selvaratnam
36. Annie Simard
37. Karl-Frédéric Vieux
38. Shian Yea Wong

ACTIVATING PROTEIN-2 (AP2) AND SPECIFITY PROTEIN-1 (SP1) ARE IMPLICATED IN THE TRANSCRIPTIONAL REGULATION OF THE CONNEXIN26 GENE IN THE EPIDIDYMIS

Cécile Adam and Daniel G. Cyr

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Connexins (Cxs) are proteins that form transmembrane channels between neighboring cells, allowing them to communicate by the diffusion of ions and small molecules (<1 kDa). Our laboratory has previously reported that Cx30.3, 31.1 and 32 are expressed in adult rat epididymis, whereas Cx26 is expressed in young animal when the epithelium is undifferentiated; suggesting a role for Cx26 in differentiation of the epididymal epithelium. The regulation of *Cx26* gene expression is unknown in the epididymis. The objective of this study was to elucidate the mechanisms regulating *Cx26* gene expression in the epididymis through the characterization of its promoter. The use of RLM-RACE revealed a single major transcription start site (tss) for *Cx26* at position -3829 relative to the ATG. Computational analysis revealed several AP-2 and SP1 binding sites located 5' to the tss. A 1.7kb fragment of the *Cx26* promoter was amplified and cloned into a vector containing a luciferase reporter gene. Several constructs were generated by deletions and transfected into a rat caput epididymal (RCE) cell line. The highest levels of luciferase activity were achieved with two constructs (-402 to +133 and -283 to +133 relative to the tss). One of them (-283 to +133) was analyzed by successive deletions, to target important regions implicated in *Cx26* regulation. A major decrease in luciferase activity was observed with the deletion of one AP-2 site (–148 to +133). A slight but consistent decrease was also observed with the deletion of the Sp1 site (-64 to +133). These results suggest that the transactivation of the *Cx26* gene is regulated by cis-acting AP-2 and SP-1 sites. The elucidation of these mechanisms will provide a better understanding not only of the regulation of Cx26 but also the processes implicated in the differentiation of the epididymal epithelium. Supported by NSERC.

4-3-3E IS AN OUTER MITOCHONDRIAL MEMBRANE SCAFFOLD WHICH BUFFERS THE ROLE OF TSPO IN STEROIDOGENESIS

Yasaman Aghazadeh[1], Martine Culty[1,3], and Vassilios Papadopoulos[1,2,3,4].

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The rate limiting step in steroidogenesis is the import of the main precursor, cholesterol through outer mitochondrial membrane (OMM). This import is hormonally regulated and is mediated by OMM multi-protein complex, the transduceosome. Translocator protein (TSPO), steroidogenic acute regulatory protein (STAR) and voltage dependent anion channel (VDAC1) are main components of transduceosome. STAR is hormonally induced and mobilizes cholesterol in the cytosol. Tspo binds cholesterol with high affinity and through its interactions with vdac1, cholesterol enters mitochondria. Regulation of steroidogenesis relies significantly on the collaboration between star, TSPO and VDAC1. However the nature and regulation of these interactions are ambiguous, suggesting the involvement of a scaffold protein. In this study, 14-3-3ε is introduced as an OMM scaffold protein that interacts with STAR, TSPO and VDAC1 in a time-depended manner upon cAMP treatment of mouse tumor Leydig cell line, MA-10 cells. STAR and VDAC1 interactions with 14-3-3ε regulate the interactions of this protein with TSPO since using peptides that block VDAC1/STAR site of interaction on 14-3-3ε, enforces 14-3-3ε-TSPO interactions and steroidogenesis, the effect observed in 14-3-3ε knock down. This induction causes a higher affinity for its exogenous ligand, PK 11195 and higher rate of interactions with VDAC1. Furthermore, using a drug targeting cholesterol binding domain on TSPO brings down steroid levels to control levels indicating that 14-3-3ε affects both cholesterol binding to TSPO and import to mitochondria through TSPO and VDAC1. The rate of steroidogenesis was also measured in organ cultures of rat testes treated with peptides that induce 14-3-3ε-TSPO interactions, indicating that under such conditions, steroidogenesis reaches levels similar to that of control testes induced with hCG for 2 hrs. Therefore, 14-3-3ε can be targeted to induce testosterone production in rats with low levels of LH.

HUMAN TESTIS STEROIDOGENESIS IS INHIBITED BY PHTHALATES

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Phthalic acid esters are known to exert potent anti-androgenic effects in the rat. In humans, studies show an inverse correlation between the concentrations of phthalates in the urine of pregnant women and the ano-genital distance (AGD) of their male infants, and between phthalate levels in the human breast milk and free testosterone levels in newborn boys. However, direct anti-androgenic effects were not found using human fetal testes in culture. Besides, only very few epidemiological studies, giving somewhat contradictory conclusions, have tackled the issue of phthalate effects in the adult: to our knowledge, the *direct* effects of phthalates on the ability of the human adult testis to produce steroids have never been assessed.

Hence, the present study aimed at investigating the possible effects of DEHP (di-(2-ethylhexyl) phthalate) and its metabolite mehp (mono-(2-ethylhexyl) phthalate) on human testicular function, using an adult testicular explant culture system originally developed in our laboratory.

Radio-immunoassays on the culture media showed a significant inhibition of testosterone production by both 10^{-5}m DEHP - which was found to be metabolized in our culture system (radio-hplc) - and 10^{-5}m MEHP, while the gross anatomy of the testis appeared to be normal. The number of germ cells undergoing apoptosis, which were caspase 3-immunolabelled and counted, did not increase with phthalate exposure. After 24hr of DEHP or MEHP treatment, neither insulin-like factor 3 (Leydig cell marker) nor inhibin B (Sertoli cell marker) production, both assayed by ELISA, were affected. The overall steroid profile for the Δ 5 and Δ 4 testosterone production pathways, assessed by GC-MS/MS, confirmed the anti-androgenic effect of MEHP, and suggested that the latter might act upstream of cholesterol transformation.

This study demonstrates that phthalate esters can exert direct and specific anti-androgenic effects on the human adult Leydig cells.

IMPROVEMENT OF PREIMPLANTATION GENETIC SCREENING (PGS) FOR ANEUPLOIDY OF SPARE HUMAN OOCYTES USING A NEW 12-CHROMSOME FLUORESCENCE *IN SITU* HYBRIDIZATION (FISH)

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Fluorescence *in situ* hybridization (FISH) has been used for decades as a principal methodology in the field of preimplantation genetic diagnosis (PGD). FISH has been routinely used in Preimplantation Genetic Screening (PGS) for screening the most frequently chromosomes involved in aneuploidy; autosomes 13, 15, 16, 17, 18, 21, 22 and gonosomes X and Y. Although new high-throughput technologies (HTP) have been recently developed such as array comparative genomic hybridization (aCGH) screen all the 23 set of chromosomes. These technologies have not been yet standardized compared to FISH in clinical applications. Taking the advantage of available aCGH data of aneuploidy, suggested that the commonly used probes in PGS might fail to detect other aneuploidies affecting preimplantation embryos. The aim of this pilot study is whether PGS strategies using FISH could be improved by re-evaluating which chromosomes are screened. Using previously published data of aCGH data, it is possible to design new FISH probe combinations that examine between 10 to12 chromosomes. We have developed a new 12-chromosome FISH test (11, 13, 15, 16, 17, 18, 19, 20, 21, 22, X, Y) that is optimized for PGS of polar bodies and corresponding MII oocytes. A total of 30 spare MII oocytes were fixed and hybridized with the 12-chromosome FISH test. Although results remain preliminary, one MII/1PB and one GV were aneuploid for newly tested Chr.11, 19 and 20 and euploid for the rest of the chromosomes. This new combination of twelve probes may significantly increase the diagnostic accuracy and efficiency rate of FISH in PGS.

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

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In the mouse embryo, the first cell differentiation occurs after the 4th division. Some 8-cell blastomeres divide asymmetrically yielding outer polar and inner apolar cells. The inner cells become the inner cell mass (ICM) in the blastocyst, while the outer cells become the trophectoderm (TE). YAP is a key transcriptional coactivator for TE/ICM specification, shuttling between the nucleus and the cytoplasm by phosphorylation. YAP nuclear localization in outer cells is essential for Cdx2 expression to specify TE fate, while YAP in inner cells is phosphorylated and sequestrated to the cytoplasm. Although outer/inner allocation of polar/apolar cells was thought to be regulated by division orientation, our live imaging analysis suggested the involvement of an active cell sorting process.

We used isolated single 8-cell blastomeres as a simple model to study this sorting process. When isolated 8-cell blastomeres divided symmetrically, two polar daughter cells reestablished cell-contact and opposed one another with a flat cell-contact. In contrast, when they divided asymmetrically, polar cells enveloped apolar cells. The enveloping process appeared to be four sequential events: 1- establishing cell-contact, 2- curving of the cell-contact, 3- thickening of the spread edges, 4- enveloping. Interestingly, YAP phosphorylation was higher in apolar cells soon after division before envelopment. These data suggested that, even before establishing the outer/inner configuration, polar/apolar cells have intrinsic differences that drive their outer/inner allocation, and simultaneously drive their fate specification through the regulation of YAP phosphorylation.

ASSESSING THE ROLE OF BETA-DEFENSIN-126 ON SPERM MOTILITY

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A crucial function of the epididymis is the establishment of a surface glycocalyx that encapsulates maturing spermatozoa. The glycocalyx may be important for regulating sperm maturation and capacitation. One of the secretory products of epididymis, β-defensin126 (DEFB126) also termed epididymal secretory protein (ESP13.2), is a dominant component of the sperm glycocalyx in macaque. Defensins are a family of cationic antimicrobial peptides. Our laboratory has shown that DEFB126 mRNA levels are highest in the caput epididymidis of human and lower in the other segments. DEFB126 mRNA levels in the caput epididymidis were 2-fold lower in non-obstructive azoospermic patient as compared with patients with proven fertility. The objective of this study was to determine if there exists a correlation between DEFB126 and sperm motility in humans. Furthermore, we intend to establish an in vitro model using the FHCE1 human epididymal cell line to establish the role of DEFB126 on sperm motility. Immunofluorescent labeling of DEFB126 indicate a greater proportion of swim-up sperm were positive for DEFB126 as compared to sperm collected from the bottom of the tube. Preliminary data comparing sperm from men with normal motility versus men with lower motility indicate that DEFB126 levels are lower in less motile sperm. An in vitro sperm motility model was established in which immotile sperm were cultured with epididymal cells. Time-dependent analyses indicate that sperm motility could be induced after 9hrs of co-culture using bicameral chambers. FHCE1 cells were also shown to express DEFB126. Experiments are ongoing to establish if DEFB126 binds to motile sperm in vitro. These preliminary data suggest that DEFB126 is associated with human sperm motility and that motility can be induced in vitro using the FHCE1 human epididymal cell line. Supported by CIHR.

THE CLAUDIN FAMILY OF TIGHT JUNCTION PROTEINS IS ESSENTIAL FOR NEURAL TUBE CLOSURE

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Neural tube closure is dependent on the differentiation of ectoderm into neural and non-neural progenitors and the coordinated morphogenetic movements of these populations of cells. Our lab has shown that members of the claudin family of tight junction proteins are differentially expressed in ectoderm prior to its differentiation into neural and non-neural progenitors and that these expression patterns are maintained throughout neurulation. To test my hypothesis that claudins are important for differentiation and for coordinating the morphogenetic movements of neural and non-neural ectoderm, I used the C-terminal domain of Clostridium perfringens enterotoxin (C-CPE) to remove a subset of claudins from the ectoderm of chick embryos during neurulation. GST-treated control embryos developed normally while GST-C-CPE-treated embryos had open neural tube defects (NTDs) and shortened anterior-posterior axes. The C-CPE-induced NTDs were classified according to their location along the anterior-posterior axis and their similarity to human defects: 7% of the embryos had an open NTD at the anterior end (anencephaly), 56% had an open NTD at the posterior end (spina bifida) and 37% had open NTDs along the entire length of embryo (craniorachischisis). In situ hybridization analysis of C-CPE-treated embryos with genes expressed in neural ectoderm (Sox2), gene expressed at the boundary between neural and non-neural ectoderm (Pax7) and genes with anterior-posterior boundaries (Otx2 and Pax6) revealed that differentiation of the ectoderm and anterior/posterior and neural/non-neural boundaries were maintained. These data suggest that claudins directly affect the morphogenetic movements required for neural tube closure but not initial differentiation of cells in neural and non-neural ectoderm.

INHIBITION OF HISTONE DEACETYLASES ENHANCES DNA DAMAGE REPAIR IN SCNT EMBRYOS

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We have previously shown that somatic cell nuclear transfer (SCNT) embryos with faster development (early cleavage) and better quality (higher cell number) have less DNA damage. We have also demonstrated that treatment with histone deacetylase inhibitors (HDACI) improve development of SCNT embryos in swine. In this study we evaluated the effect of the HDACI Scriptaid on DNA-damage repair during early development of SCNT swine embryos. Reconstructed embryos were treated with 500nM of Scriptaid for 15h starting after nuclear transfer and oocyte activation. Embryos were produced using control fibroblast cells or with fibroblasts that were exposed to ultraviolet light (UV treatment; 10 seconds) to induce DNA damage. Increased UV-induced DNA damage was confirmed in donor cells by assessing the presence of phosphorylated histone H2AX (ɣH2AX) and 53BP1 using immunocytochemistry and western blotting after 24h from UV exposure. In SCNT, the cleavage rate was not different (82% vs. 76%; $p>0.05$) but the blastocyst rate was reduced (37% vs. 3%; $P<0.05$) by UV treatment of nuclear donor cells. Higher incidence DNA damage, as assessed by the number of ɣH2AX foci, was observed in 1-cell (7.6 vs. 56.9 foci; $P<0.05$) and 2-4 cell (7.9 vs. 44.2 foci; $P<0.05$) stage embryos produced from UV-treated cells. Interestingly, Scriptaid treatment increased development (3% vs. 17.7%; $P<0.05$) and reduced DNA damage at 1-cell (56.9 vs. 41.4 foci; $P<0.05$) and 2-4 cell (44.2 vs. 28.8 foci; $P<0.05$) stage in SCNT embryos derived from UV-exposed cells. These findings indicate that DNA-damage in donor cells is an important component affecting development of SCNT embryos and that HDACI treatment enhances DNA-damage repair during cell reprogramming in SCNT swine embryos.

OVEREXPRESSING DNMT3L LEADS TO DIFFERENTIAL REPRODUCTIVE OUTCOMES UPON MALE AND FEMALE GERMLINE TRANSMISSION IN MICE

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Epigenetic reprogramming of DNA methylation patterns during gametogenesis occurs in a sex-specific manner. DNA methylation is erased during fetal development in male and female primordial germ cells. DNA methylation is mostly reacquired prenatally in males, but entirely postnatally in females. DNA methyltransferase 3-like (DNMT3L) has no intrinsic methyltransferase activity, but cooperates with DNMT3a in *de novo* methylation and is essential for normal fertility. This study sought to determine the reproductive outcome of overexpressing DNMT3L in male and female germ cells. A construct was made containing *Dnmt3L* under control of a truncated Elongating Factor 1-α promoter, expressed specifically in gonads. Transgenic mice were produced: 3transgenic male and 7 transgenic female founders were mated with wild-type animals. Transgene copy number was determined by qPCR and ranged from 3 to 263 copies. Out of 118 animals born to transgenic male founders, 116 survived to adulthood (2% loss), whereas only 94 out of 132 animals born to transgenic females survived (29% loss). Male founders transmitted the transgene to 53 out of 118 animals (45%), whereas female founders only transmitted the transgene to 21 out of 94 animals (22%). Testis histology was similar in transgenic and wild-type littermates. Ovarian histology and cellular transgene expression are being examined. Our results suggest that DNMT3L overexpression has more deleterious effects on female than male germ cells. The differential timing of methylation acquisition in males and females might be the cause. Further transgene transmission studies across multiple generations, and epigenetic profiling of gametes are ongoing.

ROLE OF UBIQUITIN LIGASE HUWE1 IN MODULATING MALE GERM CELL DEVELOPMENT

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Introduction: The omnipresent ubiquitin proteasome system is an ATP-dependent enzymatic machinery that conjugates ubiquitin on substrates to regulate their functions in physiological processes or to lead them to degradation by the 26S proteasome, thereby affecting protein turnover. We have identified an ubiquitin ligase called Huwe1 in the testis, shown to ubiquitinate all the histones in vitro and to affect cerebellar differentiation and development in the brain. In the testis, Huwe1 was highly expressed in the spermatogonia and pachytene spermatocytes. I hypothesize, Huwe1 affects male germ cell proliferation, differentiation and development as it does in the brain. **Aim:** To investigate the role of Huwe1 in the testis by its germ cell inactivation. **Results:** DdX4-cre mice expressing recombinase in primordial germ cells were mated with female mice carrying a floxed Huwe1 allele. The knockout mice (KO) were infertile with smaller testis, complete absence of germ cells, abnormal tubular morphology and a Sertoli cell only phenotype. Testicular analysis at various developmental time-points revealed that the germ cell number fell drastically at 6 days postnatally with no significant change in proliferation or apoptosis. There was deregulation of spermatogonial stem cell self-renewal and differentiation. Inactivating Huwe1 at a later stage in development using Stra8-Cre mice rendered the KO subfertile with smaller testes, several abnormal tubules and decreased sperm count. **Conclusion:** Inactivating Huwe1 early in development led to a defect in spermatogonial self-renewal and differentiation in the first wave that affected germ cell development subsequently, while a later stage inactivation led to defects possibly in meiosis or spermiogenesis.

DIFFERENTIATION OF GERM CELLS EN ROUTE TO FORM THE HERMES BODY OF EPIDIDYMAL SPERM

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The hypothesis that the germ cell Golgi apparatus appears in a structure previously named the cytoplasmic droplet but renamed by us as the Hermes body (the winged messenger transforming immotile to motile sperm) of spermatozoa of the epididymis was tested with antibodies to 60 different proteins. These antibodies were selected from a proteomic characterization of isolated testis Golgi fractions and Hermes bodies from epididymal spermatozoa that characterized 2064 proteins quantitatively in these isolated structures. From the detailed analysis by immunohistochemistry of in situ testis and epididymis, we found that 19 of these antibodies localized to Golgi apparatus of germ cells of the testis and 13 of these also localized to the Hermes body of epididymal spermatozoa. An additional 34 antibodies localized to proteins found only in the Hermes bodies in situ. Since germ cells of the testis undergo differentiation across 14 stages of the cycle of the seminiferous epithelium, we could uncover new Golgi apparatus markers, which occurred during the 19 steps of spermatid differentiation, termed spermiogenesis. This enabled us to define for the first time Golgi markers for spermatids at different phases of spermiogenesis when they form the acrosome, after its completion and then at step 19 when the Hermes body is formed and segregates from residual bodies. Besides demonstrating a Golgi relationship to the Hermes bodies in epididymal sperm we also uncovered a major function the Hermes body in the acquisition of sperm motility through these studies.

ANDROGENS REGULATE THE SECRETION OF PROSAPOSIN IN RAT EPIDIDYMIS.

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Mammalian spermatozoa become functionally mature as they pass through the epididymis, acquiring the ability to move forward, undergo acrosome reaction, bind to and penetrate the oocyte vestments and attain syngamy. The newly formed spermatozoa are surrounded by plasma membranes that are modified during epididymal maturation. The remodelling process includes the uptake of epididymal glycoproteins and modification of lipid composition. Recently, we demonstrated that prosaposin (PSAP), the precursor of saposins A-D, is secreted by the epididymal epithelium. In addition, immunofluorescence and flow cytometry indicate that the enzyme Arylsulphatase A (ARSA) and PSAP are present on the heads of caudal epididymal sperm. This observation suggests that acquisition of ARSA and PSAP increase during epididymal transit. ARSA modifies sulphatide, a process that requires prosaposin (PSAP) or its derived saposin B. The major sulfoglycolipid of the sperm plasmalemma is a sulfogalactosylglycerolipid (SGG), suspected to be a substrate of ARSA/PSAP. Based on these findings and considering the androgen dependence of the epididymis, we examined the regulation of PSAP secretion in epididymal fluids. To this effect we used control and castrated rats followed or not by testosterone replacement. Samples were analyzed by electrophoresis and immunoblotting under reducing or non-reducing conditions. We observed the presence of PSAP oligomers (250 kDa) in the epididymal fluids of the three regions, demonstrated that castration decreases monomeric PSAP (70 kDa) and increases oligomeric PSAP. These effects were reversed by testosterone replacement, indicating that androgens regulate the secretion of PSAP in the epididymal epithelium.

THE ROLE OF CLAUDIN-10 IN PATTERNING THE LEFT-RIGHT AXIS

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Asymmetric organ positioning within the limited space of the body cavity is critical for normal physiology. The origin of this asymmetry is initiated early during development and activates an evolutionarily conserved molecular cascade that defines the left and right sides of the embryo. We have identified a role for the tight junction molecule, *Claudin-10*, in asymmetric organ positioning in the chick. Claudins are components of tight junction, where they function to regulate the movement of ions within the paracellular space and link the tight junction to the actin cytoskeleton via interactions between their cytoplasmic tails and proteins in the cytoplasmic plaque.

We find that *Claudin-10* is asymmetrically expressed on the right side of Hensen's node, a critical site where bilateral symmetry is broken. We demonstrate that overexpression of Claudin-10 on the left side, or knockdown of endogenous Claudin-10 on the right, randomizes the direction of heart-looping, the earliest morphological sign of disrupted left-right patterning. Furthermore, expression of genes in the left-right patterning cascade is randomized in manipulated embryos. Mutagenesis of charged residues in the domain determining ion permeability properties did not affect the function of Claudin-10 in asymmetric morphogenesis. However, mutation of two sites in the cytoplasmic tail abolished the ability of Claudin-10 to randomize the direction of heart looping in gain-of-function studies. We find that asymmetric Claudin-10 expression is required for normal patterning of the left-right axis, and that interactions with proteins at the cytoplasmic plaque are involved in its role in axis patterning.
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MONITORING TRANSCRIPTIONAL ACTIVITY IN LIVING OOCYTES AND EARLY EMBRYOS

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The development of an objective, non-invasive method of predicting oocyte and embryo competency with high accuracy is valuable to the improvement of *in vitro* culture and to the advancement of assistive reproductive technologies. One approach is to take advantage of the discontinuity of RNA transcription in the oocyte and the early embryo. Detection of the normal progression of transcriptional activity in these cell types may provide insight into their developmental competence. We aimed to determine if this is possible using the transgenic TCF/Lef-LacZ mouse strain. Oocytes from these mice exhibit a temporal pattern of β-galactosidase expression throughout development. Using fluorescein-di-galactoside (FDG) and confocal microscopy, we show that this temporal pattern follows the pattern of transcriptional activity in the oocyte and early embryo. Through *in vitro* culture of ovarian follicles and granulosa-oocyte complexes, we show continued growth of oocytes after FDG live-staining and imaging. Using 5-ethynyl uridine incorporation, we confirm the relationship between the presence of the reporter signal and active transcription in the oocyte and the early embryo. Furthermore, growing oocytes cultured in granulosa-oocyte complexes lose β-galactosidase expression after 48 hour culture in α-amanitin, a known transcriptional inhibitor, confirming that expression of the reporter protein is regulated at the level of transcription. Thus, TCF/Lef-LacZ mice together with FDG as a vital stain appear effective for monitoring changes in transcriptional activity in the developing oocyte and embryo. This ultimately shows promise towards the creation of an in vitro, non-invasive predictor of developmental competence in living oocytes and embryos.

REGULATION OF GROWTH HORMONE RECEPTOR EXPRESSION BY MIRNAS.

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The pleiotropic actions of Growth Hormone (GH) result from binding to its receptor (GHR) on target cells and the subsequent stimulation of multiple intracellular signaling pathways, leading to changes in gene expression, differentiation and metabolic activity. Clinical studies have associated over- or under-expression of GHR with effects on childhood growth, obesity, cancer and diabetes, suggesting that GHR levels must be tightly regulated at every stage of life. Previous studies have focused on promoter (5'UTR) regulation of *GHR* gene expression. In the present study, we are investigating the role of miRNAs at the 3'UTR: miRNAs are small (19-21nt) noncoding RNAs that regulate gene expression through enhancing mRNA degradation or inhibiting translation. In the last decade, a critical role for miRNAs has been demonstrated in many chronic diseases, including cancer.

We initially used multiple *in silico* prediction tools to define putative miRNA-binding sites within the *GHR* 3'UTR and prioritized a subset based on hybridization energy, conservation across species, and reports that link the miRNAs to GHR-related physiological or pathophysiological activities. To test these sites, we created a Luc-*GHR* 3'UTR luciferase reporter vector and screened for miRNA activity: miR-16 ($p<0.05$), miR202 ($p<0.01$), miR129-5p ($p<0.01$) and miR142-3p ($p<0.01$) showed significant inhibitory effects. Studies are underway to determine the specificity of their sites (site-directed mutagenesis) as well as to examine their effects on endogenous *GHR* mRNA (qPCR) and protein (western blot) expression levels. Future goals are to determine the role of these miRNAs in GHR-related pathophysiologies and their potential use as therapeutic agents.

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POLYA MRNA TAIL LENGTH DURING OOCYTE MATURATION

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Oocytes are transcriptionally inactive during meiotic maturation. In mice, transcription resumes at the 2-cell stage. Hence the oocyte must rely on the highly regulated recruitment of specific maternal mRNAs to undergo the processes of maturation, fertilization and early embryogenesis. We and others have previously shown that some transcripts have a variable length of polyA tail during these processes. We propose two patterns of polyA tail modification: for certain mRNAs such as *Ccnb1*, the polyA tail is lengthened and remains stable. For others such as *Slbp*, the polyA tail is shortened around 8h after the initiation of maturation. In the present study, we wish to correlate changes in the lengths of polyA tails with the silencing and degradation of these transcripts after they have served their roles in maturation. PolyA tail lengths will be analyzed at the different stages of maturation using RNA Ligation-PolyA Test (RL-PAT). The timing of degradation will also be determined by RT-qPCR. Preliminary results show a decrease in the expression of *Actb, Emi1, Erk3, Plat* and *Mos* after the first meiotic division. These results could help identify a precise time point during maturation when specific mRNAs are degraded after having been stored for an extended period during early oogenesis. This would potentially allow for the identification of a molecular basis for the timing of this highly regulated degradation process.

THE ROLE OF UTERINE PORCUPINE IN IMPLANTATION AND MOUSE FERTILITY

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The Porcupine gene encodes a protein of the conserved O-acyl transferase superfamily required for palmitoylation of WNTs in the ER; a modification necessary for their secretion. The secretion of various WNTs by the uterus and the embryo has been found to be essential in order to create the embryo-uterine cross talk which coordinates the implantation process. We have shown that porcupine is expressed in the luminal and glandular epithelial cells of the uterus. The expression level of the gene increases shortly after mating but begins to decline at E3.5. In order to address the function of Porcupine in the implantation process, we have generated a uterine specific deletion using a progesterone receptor-Cre (PR-Cre) system. Heterozygous uterine specific deleted Porcupine females appear to be cycle regularly but most heterozygous females are sterile. These females exhibit hyperplasia of the uterine glands and form what appear to be uterine cysts. Homozygous Porcupine female uteri are significantly smaller and under-developed and are devoid of endometrial glands. The homozygous Porcupine females are infertile and their uterus resembles the uterus of postnatal day 15 pups. This phenotype resembles the uterine specific deletion of Wnt7a. In attempt to rescue the mutant phenotype, Wnt7a was injected into the uterus and through serial IP-injections. Injected females exhibited extensive branching and coiling of the luminal epithelium, marking the re-establishment of uterine glands. Our results demonstrate that Porcupine is necessary for uterine glandular epithelial development.

OSR1$^{+/-}$ MICE: A MODEL OF URINARY TRACT DEFECTS

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Congenital Abnormalities of the Kidney and Urinary Tract (CAKUT) are a cause of chronic kidney failure and encompass a spectrum of phenotypes including vesico-ureteric reflux (VUR), the retrograde movement of urine from the bladder to the kidney, duplex systems and/or urinary tract (UT) obstruction. Our laboratory has previously identified the C3H/HeJ (C3H) mouse as a model of fully penetrant VUR compared to the non-refluxing C57Bl/6 (B6) mouse strain and identified a 22Mb susceptibility locus on the proximal end of chromosome 12: *Vurm1*. The *Vurm1* locus contains many candidate genes, including Odd Skipped Related 1(*Osr1*), which is expressed in the intermediate and undifferentiated metanephric mesenchyme. Homozygous null mice have been previously reported to die at birth from cardiac defects and the absence of kidneys. We found that heterozygous *Osr1*$^{+/-}$ newborn pups on a B6 background exhibit VUR, 5/19 (26.31%) with no gender effect compared to almost no reflux 1/25 (4%) in wild-type mice ($P<0.05$). Other urinary tract abnormalities including ureter duplication (n=5) and megaureter (n=1) are also observed in heterozygous mice, but there is no evidence of any renal malformation based on kidney surface area and sections stained with hematoxylin and eosin. Newborn *Osr1*$^{+/-}$ pups from crosses between C3H and *Osr1*$^{+/-}$ mice exhibit a 9% incidence of VUR. Furthermore, no coding mutations were found when *Osr1* was sequenced in the C3H mouse. This data suggests that while *Osr1* may not be the gene conferring VUR susceptibility in the C3H mouse, *Osr1* haploinsufficiency does have a critical impact on urinary tract development.

MMACHC IS REQUIRED DURING THE PERI-IMPLANTATION PERIOD IN MOUSE EMBRYOS.

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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 cblJ*,and *mut*. These inborn errors can lead to abnormal development of the neural tube and other birth defects in humans, and result in elevation of either homocysteine or methylmalonic acid, or both, in blood and urine. The *cblC* disorder (OMIM 277400), in which there is both hyperhomocysteinemia and methylmalonic acidemia, is the most frequent inborn error of vitamin B_{12} metabolism and is due to mutation in the *mmachc* gene. Clinical features of patients can include hematological, neurological, and ophthalmological findings, along with developmental delay. The aim of this project is to determine the expression and function of *Mmachc* during mouse development in hope of creating a mouse model for human cblC. *In situ hybridization* analysis showed cell type and tissue-specific expression pattern of *Mmachc* in heart and also in endothelial cells of blood vessels in developing embryos, suggesting an important role for *MMACHC* during development of the circulatory system. The gene-trap mouse line showed that *Mmachc* heterozygous mutant mice were fertile and viable. However, we found that heterozygous mutant mice at E11.5 had decreased levels of MMACHC proteins and decrease in ERK1/2 phosphorylated proteins, compared with the wild-type mice. Homozygous mutant *Mmachc* embryos were only obtained at embryonic day 3.5.Our results suggest an early requirement of *Mmachc* gene during the peri-implantation processes.

UNCOVERING THE FUNCTION OF TMED2 DURING TROPHOBLAST DIFFERENTIATION

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TMED2 is a member of the p24 family of proteins involved in vesicle transport between the endoplasmic reticulum (ER) and Golgi. TMED2 localizes predominantly in the ER Golgi Intermediate Compartment, where it is proposed to function as a receptor for specific protein cargoes and coat proteins. We have shown that TMED2 is expressed and required in the mouse placenta. In fact, mouse embryos with mutations in Tmed2 fail to form syncytiotrophoblast cells, a cell type, which is critical for labyrinth placenta function. Expression of TMED2 is conserved in human placentas, and we reported expression of TMED2 between 5.5 and 40 weeks of gestation in all trophoblast cell types, including the syncytiotrophoblast. Moreover, early in gestation TMED2 was more highly expressed in cytotrophoblast cells versus syncytiotrophoblast. In addition, we found that TMED2 was more highly expressed in the BeWo choriocarcinoma cell line, which differentiates to form syncytiotrophoblast, when compared to JEG-3 cells, which do not form syncytiotrophoblast. The Aim of this study is to determine if TMED2 is sufficient or required during syncytiotrophoblast differentiation. Methods: in this study we generated JEG-3 cell lines with stable expression of eGFP, and N-terminal tagged eGFP-eGFP-TMED2. We used Immunohistochemistry, PCR and western blot analysis to examine differentiation of the stably transfected cell lines. Results: We found that ectopic expression of TMED2 in JEG-3 resulted in a significant increase of syncytiotrophoblast formation when compared to normal or eGFP expressing JEG-3cells. Conclusion: Our preliminary results suggest that TMED2 is sufficient to induce fusion in JEG-3 cells. In the future we will generate BeWo cell lines with knockdown of TMED2 in order to determine if this gene is also required for syncytiotrophoblast formation.

PROTEOMICS CHARACTERIZATION OF THE CYTOPLASMIC DROPLET OF EPIDIDYMAL SPERM

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Spermatozoa of the epididymis contain a cytoplasmic bulge along the flagellum called the cytoplasmic droplet (CD), which reveals flattened cisternal membranes. We hypothesize that the CD may be relevant to sperm maturation and that the internal membranes of this structure are derived from the Golgi apparatus of testicular germ cells. The protein makeup of the isolated CD fractions of epididymal sperm (CDF) was compared to proteins of testis germ cell Golgi apparatus (TGF) using subcellular fractionation. Quantitative tandem mass spectrometry revealed that 765 proteins were shared between the two structures. Of the 765 proteins, 68 proteins were examined by immunohistochemistry (IHC) in the testis and epididymis. Quantitatively, the most abundant CD proteins were those for glucose transport (glut3) and metabolism (hexokinase 1) and both were localized in situ to epididymal sperm. In addition, the CD contained proteins characteristic of ER and Golgi apparatus. By contrast, the TGF enriched in germ cell Golgi apparatus revealed that the most abundant protein as a novel putative glycosyl transferase (GL54D), as well as other established germ cell Golgi glycosyl transferases again verified by IHC in situ. Golgi proteins conserved between the CDF and TGF and verified by IHC included those proteins involved in structure and identity, including the P24 transmembrane proteins, the ATPase NSF, the GTP exchange factor −GBF1, and COPI coatomer proteins. We conclude that the CD is a unique structure whose membrane components and molecular constituents work as a molecular machine to coordinate energy production coincident with the acquisition of sperm motility and functions related to sperm maturation.

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

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During vesicular transport between the endoplasmic reticulum and the Golgi, members of the transmembrane emp24 domain (TMED) protein family form hetero-oligomeric complexes that facilitate protein cargo transportation and secretion. In our laboratory, we are studying the function of one member of the TMED protein family, TMED2, in mouse placental labyrinth development. Formation of the mouse labyrinth layer requires proper interactions between two extraembryonic tissues, the allantois and the chorion; and is essential for nutrition, waste, as well as hormone exchange between fetal and maternal circulation. We have shown that *Tmed2* is expressed in both allantois and chorion and is required for normal labyrinth layer formation. In *Tmed2* mutants, expression of syncytiotrophoblast cell marker, *Gcm1* is reduced. We hypothesized that TMED2 is essential in the chorion for normal interaction between the allantois and chorion- a critical step in placental labyrinth layer development. To test this hypothesis, we have generated an ex-vivo allantois and chorion recombination model. In our model, we recapitulated the early events of labyrinth layer development: chorioallantoic attachment, fusion of the mesothelium and allantois, and chorionic trophoblast differentiation. We used in situ hybridization and immunohistochemistry to confirm the chorioallantoic attachment event and to monitor development of labyrinth layer in the chimeric explants. Our preliminary results showed that the chorioallantoic attachment is required for maintaining expression of syncytiotrophoblast cell marker, *Gcm1*. Our data suggest a previously unknown requirement for chorioallantoic attachment for maintenance of the spongiotrophoblast cell marker, *4311* in *ex-vivo* cultures. In the future we will use combinations of wild type and *Tmed2* null chorion and allantois in these ex-vivo cultures to determine which tissue TMED2 is required in for normal chorioallantoic attachment and to identify the protein cargoes transported by TMED2 during labyrinth layer formation. 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 early pregnancy loss and intrauterine growth retardation.

ROLE OF ATAD3 IN INTER-ORGANELLE INTERACTIONS IN HORMONE-INDUCED LEYDIG CELL STEOIRDOGENESIS.

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Steroid formation is a multi-step process initiated with the transport of the cholesterol from intracellular stores into the outer mitochondrial membrane (OMM) through a complex termed the transduceosome, in which cytosolic proteins interact with OMM proteins. From there, cholesterol reaches the cytochrome P450 CYP11A1 enzyme in the inner mitochondrial membrane (IMM) through a bioactive 800-kDa protein complex present at the outer/inner membrane contact sites, the steroidogenic metabolon. CYP11A1 converts cholesterol to pregnenolone. Cholesterol transfer from OMM to CYP11A1 is the rate-limiting step of this process. Using BN-PAGE in tandem with mass spectrometry analysis, the AAA+ ATPase ATAD3 was identified to be part in the later complex. ATAD3 is anchored in the IMM and enriched at OMM-IMM contact sites. ATAD3 RNA silencing in MA-10 Leydig cells revealed the critical role of this protein in mitochondrial cristae organization and the hormone-stimulated steroid formation. Moreover, knocking down ATAD3 resulted in a decrease in OMM-IMM contact site formation and endoplasmic reticulum (ER)-mitochondria physical interactions but had no effect on 22R-hydroxycholesterol-supported steroid formation suggesting a role for this protein in cholesterol transfer to CYP11A1. The C-terminal region of ATAD3 in the matrix contains the ATP-binding domain, named *Walker A*. Mutation of the *Walker A* domain resulted in a reduction in Leydig cell steroid formation suggesting a role of ATP in the function of ATAD3 in cholesterol transport. The N-terminus of ATAD3 contains 50 amino proposed to form an alpha-helix that drives the insertion of the protein back into OMM and associated organelles, such as ER. Indeed, ATAD3 has been found in mitochondria associated membranes (MAMs), regions of close apposition between OMM and ER. These structures would allow the transfer of the substrate cholesterol into mitochondria. We hypothesized that ATAD3 functions as a bridge between the mitochondrial membranes and as a contact point between organelles, in the MAMs. Deletion of the ATAD3 N-terminus resulted in the reduction of hormone-stimulated steroidogenesis suggesting a role of ATAD3 in the mitochondria-ER contact site formation. Taken together these results suggest the presence of inter-organelle communications in Leydig cells, mediated by ATAD3, which appear to be crucial for the transfer of cholesterol into mitochondria for steroidogenesis.

THE HISTONE H3 LYSINE 4 DEMETHYLASE, KDM1A IS ESSENTIAL FOR MEIOTIC PROGRESSION AND THE SURVIVAL OF SPERMATOGONIAL STEM CELLS.

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Spermatogenesis is a highly complex cell differentiation process fueled by spermatogonial stem cells (SSCs). Cell fate decision in the transition from an SSC to a differentiating cell involves gene expression changes likely to be under specialized epigenetic control. Epigenetic mechanisms governing gene expression involve histones and their modifiers which add and remove permissive or repressive marks from histone tails. The histone demethylase KDM1A removes gene activating dimethylation on histone H3 at lysine 4 (H3K4m2). KDM1A associates with the chromatin modifier histone deacetylase 1 (HDAC1), which removes gene activating histone H3 acetylation, forming a protein complex that induces gene silencing. As *Kdm1a* is preferentially expressed in the SSCs, we hypothesized that it serves essential functions in the epigenetic regulation of SSCs biology. To determine its function in SSCs we generated mice with a conditional knockout of *Kdm1a* (cKO) specifically in spermatogonia. KDM1A is essential for spermatogenesis as adult cKO were sterile with a total loss of germ cells. At PND 6, spermatogonia were present in the cKOs, however, by PND 10 few potentially meiotic cells were detected, none of which displayed normal spermatocyte morphology. Analysis of synaptonemal complex formation revealed that some cKO cells initiated chromosome condensation but chromosome synapsis failed. From PND 10 to 18 the number of spermatogonia in the cKO testes decreased dramatically with few germ cells remaining at PND18. To determine specificity of the knockout, H3K4m2 and H3 acetylation were assessed. At PND 6, the cKO spermatogonia presented higher levels of both gene activating marks. These results show that KDM1A targets essential genes governing spermatogonia cell fate, survival and meiotic progression.

EFFECTS OF BROMINATED FLAME RETARDANTS ON HUMAN GRANULOSA CELLS

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Brominated flame retardants (BFRs) are incorporated into consumer products to prevent flame propagation. They leach out into the environment and are absorbed by inhalation and ingestion. The detection of BFRs in human follicular fluid is associated with pregnancy failure, raising serious questions regarding the effects of BFRs on reproductive health, specifically on ovarian function. Here, we evaluated the effects of BFRs on a human immortalized ovarian granulosa cell line (KGN). KGN cells were exposed for 48 hours to a range of 0-127 µM of a mixture reflecting the relative composition of the major class of BFR congeners, the polybrominated diphenyl ethers (PBDEs), in North American breast milk. At the highest concentration, there was a significant reduction of 40% in the numbers of cells/well. Metabolic activity, measured using the MTT assay, was increased by exposure to 73 µM of the PBDE mixture and sharply reduced at the highest concentration. A Click-it Edu assay revealed that cell proliferation was also significantly affected by PBDE treatment. Progesterone and estradiol concentrations in the conditioned culture media were reduced 20 and 3 fold, respectively, with increasing concentrations of the PBDE mixture starting at 5 µM. Our results reveal that PBDEs disrupt steroidogenic activities and are cytotoxic to KGN cells at high concentrations. *Supported by grant RHF100625 from the IHDCYH/CIHR. PLCL has a postdoctoral fellowship from the FRQS.*

ABERRANT DNA METHYLATION PATTERNS IN BALB/C SPERM AS A RESULT OF MTHFR DEFICIENCY IN COMBINATION WITH OR WITHOUT FOLATE DEFICIENCY AND SUPPLEMENTATION

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High expression of 5,10-Methylenetetrahydrofolate reductase (MTHFR), an enzyme involved with the transfer of methyl groups in the folate pathway, in murine male germ cells concomitant with acquisition of *de novo* DNA methylation patterns suggests that both MTHFR and the folate pathway may play key roles in sperm DNA methylation acquisition. We investigated the individual effects of MTHFR deficiency in mice heterozygous for a targeted mutation in the *Mthfr* gene, with or without dietary folate supplementation/deficiency, on sperm methylation profiles in BALB/c mice. At weaning age, mice were placed on one of three amino acid controlled diets for one year: a control diet (CD) reflecting the recommended amount of folic acid, a 20-fold folate supplemented diet (FS) or a 7-fold deficient diet (FD). Both *Mthfr* wildtype (+/+) and *Mthfr* heterozygous (+/-) mice were randomly assigned to diet groups (n=4). Following treatments, sperm were processed for genome-wide DNA methylation analysis by reduced representation bisulfite sequencing. When comparing to sperm methylation in *Mthfr+/+* mice, we observed 362, 872, and 263 hypomethylated sites and 37, 198, and 157 hypermethylated sites for mice on CD, FD, and FS diets respectively, in *Mthfr+/-* mice. As expected, a reduction in MTHFR was more strongly coupled to hypomethylation of DNA than hypermethylation. However, when comparing the two genotypes, FD unexpectedly accentuated DNA hypermethylation (5.3-fold vs CD) as compared to hypomethylation (2.4-fold vs CD). Interestingly, when compared to CD, FS diminished the levels of sperm DNA hypomethylation (.7-fold) but increased the levels of hypermethylation (4-fold). Thus, while FS provides a partial amelioration of the hypomethylation, it induces significant hypermethylation. Methylation changes are being validated by pyrosequencing and require further detailed analysis of the specific sites affected as well as the functional significance as defined by assessment of progeny.

TRICELLULIN IS IMPLICATED IN THE TIGHT JUNCTIONS OF THE BLOOD-EPIDIDYMIS BARRIER AND IS RELATED TO BASAL CELL PROJECTIONS

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Tricellulin is a tight junction protein implicated in tricellular junctions. Epididymal tight junctions of the blood-epididymis barrier (BEB) protect sperm from the immune system and help create a luminal environment necessary for sperm maturation. Recent studies suggested that basal cells are implicated in the BEB. While basal cells express Claudin1 (CLDN1), there is no information regarding the potential architecture or regulation of basal cell-principal cell tight junctions. The objective of this study was to explore the expression and localization of tricellulin in rat epididymis in relation to basal-principal cell interactions and other junctional proteins. Tricellulin protein levels were similar in all segments of the adult epididymis and was localized to the apical area of the BEB. Postnatal development studies showed that tricellulin levels increased with age and its localization varied. At day 14, tricellulin was cytoplasmic throughout the epididymis, while at day 21 tricellulin localized to the lateral margins of the plasma membranes of adjacent cells. By day 42, tricellulin was apically localized along the plasma membrane while at day 56, the immunostaining was more intense and similar to the adult. Co-localization studies with occludin indicated that in the initial segment, occludin and tricellulin did not co-localize but rather localize to different areas of the lateral margin along the apical junctional complex; in the caput and corpus epididymidis, however, both proteins co-localized to apical tight junctions. In the cauda epididymidis, occludin and tricellulin did not consistently co-localize, suggesting that the composition of tight junctions in the cauda may vary. To assess whether or not these differences were due to the presence of basal cells, co-localization studies of tricellulin and KRT5 (cytokeratin5), a marker of basal cells, were performed. KRT5 co-localized with tricellulin in areas where basal cell projections occur suggesting tripartite cellular junctions between basal and principal cells. Tricellulin knock-down studies using siRNA and epididymal RCE cells resulted in decreased levels of both CLDN1 and occludin while ZO-1 and E-cadherin levels appeared unchanged. These results indicate that tricellulin is implicated in tight junctions of the BEB and that in the cauda it is implicated in basal-principal cell interactions. Tricellulin also appears to exert regulatory effects on CLDN1 and occludin. Supported by CIHR.

SIGNALING PATHWAY CROSSTALK IN NEONATAL GONOCYTE AND EMBRYONAL TERATOCARCINOMA CELL DEVELOPMENT

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Male reproduction depends on the proper development of neonatal gonocytes, the precursors of spermatogonial stem cells. It has been suggested that failure of this process may lead to testicular germ cell tumor formation. Thus, understanding gonocyte development may help us better understand the origins of this pathology. We have previously shown that gonocyte proliferation requires platelet-derived growth factor (PDGF)-BB and 17β-estradiol, and MEK1/2 activation. We identified a variant form of PDGF receptor β (V1-PDGFRβ) that was increased during retinoic acid (RA)-induced gonocyte differentiation. V1-PDGFRβ was also expressed during the RA-induced differentiation of mouse F9 embryonal teratocarcinoma cells. F9 cells differentiate into parietal endoderm, but also express Stra8 (premeiotic germ cell marker), in line with being at a stage prior to somatic-germ lineage specification. Our goal was to study the role of PDGFRs in gonocyte and F9 cell differentiation. Northern blot analysis showed that neonatal testes express PDGFR α and β variants. RA increased expression of PDGFRα and β variants in gonocytes and F9 cells. The identity of PDGFRα variants in F9 cells was confirmed by mass spectrometry. Overexpression of V1-PDGFRβ in gonocytes increased Stra8 expression and the percentage of differentiating gonocytes, but did not prevent cell proliferation. In both cell types, the inhibition of PDGFR tyrosine kinase activity reduced RA effects on mRNA expression of differentiation markers, suggesting that one or more PDGFR forms needs to be activated. MEK1/2 was activated in RA-stimulated F9 cells and its inhibition blocked differentiation towards the somatic lineage, shown by Collagen IV and Laminin B1 expression, while increasing Stra8 mRNA levels. This suggests that MEK1/2 might act as a switch for lineage specification in F9 cells. In contrast, RA-induced gonocyte differentiation, assessed by Stra8 mRNA expression, was independent of MEK1/2, but was inhibited by blocking SRC, JAK2, and STAT5 activities, indicating their possible role in gonocyte differentiation. These results suggest that neonatal gonocyte and F9 cell differentiation are regulated through crosstalk between RA and PDGFRs using different downstream pathways. It will be interesting to find whether these variants are similar to the PDGFRα variant mRNAs previously identified in testicular germ cell tumors.

EFFECT OF CELL MEMBRANE PERMEABILIZATION AND HYPEROSMOTIC TREATMENT BEFORE NUCLEAR TRANSFER ON DEVELOPMENT OF SCNT SWINE EMBRYOS

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The success of somatic cell nuclear transfer (SCNT) involves dramatic reprogramming of the transferred chromatin in the host cytoplast in order to express genes required for proper development of the reconstructed embryo. In a small proportion of attempts, the host cytoplast appears to promote sufficient remodeling of the transplanted chromatin. However, most of the SCNT embryos exhibit insufficient reprogramming and abnormal development. Previous studies from our laboratory have shown that cell membrane permeabilization using streptolysin-O (1 µg/mL at 38.5°C) for 30 min followed by exposure to Tris-NaCl buffer (10 mM Tris-HCl, 0.5 mM MgCl2, 0.7 M NaCl, 1 M sucrose) for 5 min resulted in the partial displacement of the linker histone H1 and DNA-binding HMG-17 protein in cultured porcine fibroblast cells. We hypothesized that displacement of the nuclear proteins would make the donor cell chromatin more malleable for reprogramming by the host cytoplast. In this study, our objective was to evaluate development and cell number in SCNT embryos produced from membrane permeabilized cells and from control cells. After the permeabilization treatment, cells were cultured for 1 h at 38.5°C in DMEM supplemented with 10% fetal bovine serum (FBS) and 2 mM CaCl2 for membrane resealing, and then transferred to DMEM with 10% FBS until nuclear transfer. The interval from cell permeabilization to nuclear transfer was 5-6 h. Control cells were maintained in DMEM with 10% FBS. In vitro matured oocytes enucleate, fused and activated using standard procedures were used as host cytoplasts and SCNT embryos were cultured in PZM-3 medium. Embryo development was assessed based on cleavage and blastocyst rates. Embryo quality was based on inner cell mass:trophectoderm ratio by differential staining with propidium iodide and Hoechst after trophectoderm cell permeabilization with Triton-X. Cell apoptosis was assessed by immunofluorescence using an antibody against cleaved caspase-3. Cleavage rate was higher (70.1% vs. 56.1%) in embryos produced with control cells but the proportion of cleaved embryos that developed to the blastocyst stage was similar between control (16.1%) and permeabilized cells (20.0%). Total cell count (34.5 vs. 40.0) and proportion of cell apoptosis (6.2% vs. 6.4%) were similar between control and permeabilized cells. Interestingly, the proportion of cells in the inner cell mass was significantly higher in embryos derived from permeabilized (25.0%) compared to control cells (17.1%), suggesting a benefit on cell reprogramming and differentiation. Although further studies are required to confirm the potential benefits of this protocol on chromatin reprogramming and normal gene function, our findings indicate that cell membrane permeabilization can be used to develop and test new procedures to promote cell reprogramming before SCNT. Supported by NSERC.

EFFECT OF HISTONE DEACETYLASE INHIBITOR TREATMENT ON INTER-ORDER PORCINE/BOVINE SCNT EMBRYOS

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Normal embryo development depends upon chromatin modifications that are required to precisely regulate the spatio-temporal expression of genes. Inter-order embryos produced by somatic cell nuclear transfer (ioSCNT) represent a valuable model to study nucleo-cytoplasmic interactions and chromatin reprogramming during early development. It has been shown that exposure to epigenetic modulators such as inhibitors of histone deacetylases (HDACi) can alter chromatin structure, gene expression pattern and increase development of SCNT embryos. In this study, ioSCNT porcine-bovine embryos were used to evaluate the effect of the HDACi Scriptaid on embryo development (cleavage and blastocyst formation), acetylation (H3K27ac) and trimethylation (H3K27m3) of the lysine 27 in the histone H3 (at 1-, 2- and 4-cell stages), and cell apoptosis (presence of cleaved caspase 3 (CC3) at the morula and blastocyst stages). SCNT embryos were produced by hand-made cloning using porcine fibroblasts cells and: a) porcine (control); b) mosaic (half porcine and half bovine); and c) bovine (ioSCNT) host cytoplasts. After reconstruction and activation, embryos were treated or not with 500nM Scriptaid for 10-12h and then cultured in PZM-3 for up to 7 days. H3K27ac and H3K27m3 patterns and the proportion of CC3 positive cells were compared between HDACi-treated and non-treated embryos of the 3 groups by assessing the immunofluorescence signal after staining with specific primary and Alexa Fluor 488 conjugated secondary antibodies. HDACi treatment increased development of control and mosaic embryos but not of ioSCNT embryos. HDACi treatment did not significantly affect the H3K27ac signal in 1-cell stage embryos from all groups. However, the H3K27ac signal was reduced by HDACi exposure in 4-cell embryos of the mosaic group and in 2-cell embryos of the ioSCNT group. Interestingly, the H3K27m3 signal was significantly increased by HDACi treatment in 2- and 4-cell embryos of the control and mosaic groups. In the ioSCNT group, HDACi increased H3K27m3 signal only in 2-cell stage embryos. The proportion of CC3 positive cells at morula and blastocyst stages was significantly reduced by HDACi treatment in the mosaic group but not in control and ioSCNT groups. In conclusion, HDACi treatment improves development of control and mosaic but not ioSCNT porcine-bovine embryos. The positive effect of HDACi treatment can be due to an increase in H3K27m3 in cleaved embryos, as well as by reducing cell apoptosis in mosaic embryos. Supported by FAPESC and NSERC.

P-31

ABSENCE OF PEROXIREDOXIN 6 IS ASSOCIATED WITH LOW SPERM CHROMATIN QUALITY AND SUBFERTILITY IN MICE CHALLENGED WITH OXIDATIVE STRESS

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Introduction: Oxidative stress (OS), the imbalance of reactive oxygen species (ROS) and antioxidant systems, is frequently associated with men infertility. At least 30% of infertile men have high levels of ROS in semen. An elevated level of DNA damage in human spermatozoa is a characteristic of human infertility. Spermatozoa from infertile men have low levels of peroxiredoxin (PRDX) 1 and PRDX6. Oxidation of PRDXs promotes inactivation of their antioxidant activity. Sperm lipid peroxidation, DNA damage and progressive motility depend on the levels of oxidized PRDX6 but not of PRDX1. The aim of this study was to determine the impact of OS on reproductive outcomes and sperm chromatin quality in the Prdx6$^{-/-}$ male mice.
Methods: Eight week old Wild type (WT) and Prdx6$^{-/-}$ males were treated intraperitoneally with tert-butyl hydroperoxide (60 □mol/kg/bw/
Control and treated WT and Prdx6$^{-/-}$ males were mated with young WT females and total number of litters and pups produced were obtained during a period of 6 months after treatment. DNA fragmentation (DFI) and DNA compaction (High DNA stainability, HDS) were determined by flow cytometry using the sperm chromatin structure assay. Sperm DNA oxidation was determined by immunocytochemistry using an anti-8-hydroxy-guanosine (8-OHG) antibody.
Results: Treated males had significantly less number of litters and pups than their non-treated controls ($p<0.01$); Prdx6$^{-/-}$-treated males have the lowest number of litters ($p<0.01$). DNA fragmentation and 8-OHG levels were higher and DNA compaction lower in Prdx6$^{-/-}$ spermatozoa than WT controls ($p<0.01$). Sperm chromatin parameters were inversely correlated with number of litters and of pups per male ($P<0.05$). Regression analyses showed that, sperm DFI and 8-OHG levels depend on the level of sperm DNA compaction. Moreover, the total numbers of litters and pups depend on HDS, DFI and 8-OHG.
Conclusions: Prdx6$^{-/-}$ males are more sensitive to OS showing lower sperm chromatin quality, and less number of litters and pups than WT controls. OS must be affecting spermatogonia because treated males did not recover normal fertility after new cycles of spermatogenesis. PRDX6 is required for maintaining sperm chromatin integrity to assure fertility in the mouse.

GLUCOSE UPTAKE IN THE GRANULOSA CELLS OF OVULATING FOLLICLES IN MICE

Melissa Pansera[1], Dana Praslickova[2], Anitha Kalaiselvanraja[1], Lisa Dupuis[1], Dayananda Siddappa[1], Yasmin Schuermann[1], Luis Agellon[2] and Raj Duggavathi[1]

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Granulosa cells of ovulating follicles undergo luteinization in response to the preovulatory luteinizing hormone (LH) surge. During luteinization, granulosa cells begin to express the steroidogenic enzymes involved in progesterone synthesis, all of which require energy for their function. In fact, LH is known to increase glucose uptake in the ovary, which may be used during the energetically demanding process of steroidogenesis. However, the mechanism by which LH increases glucose uptake has not been thoroughly explored. We hypothesized that LH increases ovarian glucose uptake by inducing glucose transporters in luteinizing granulosa cells. To test this hypothesis, we first determined the expression pattern of the four best characterized glucose transporters, Slc2a1-4, in granulosa cells. Cells were collected at time-points representative of follicular and luteal development during superovulatory treatment (using eCG and hCG) in immature (~23 d old) mice. There was a dramatic increase ($P < 0.001$) in the mRNA abundance of Slc2a1 at 4h after hCG stimulus. On the other hand, the mRNAs of Slc2a2, 3 and 4 were undetectable by real-time PCR at all the time-points tested. Next, we examined if increased mRNA abundance of Slc2a1 in granulosa cells was associated with an increase in glucose uptake. We determined glucose uptake in granulosa cells collected at 0h and 4h of hCG stimulus. Cells were seeded onto a 96-well plate, allowed to attach for 2 hours and were then treated with a fluorescent-labeled deoxyglucose analog, 2-NBDG, for 15 minutes. Fluorescence was measured using a microplate reader. There was significantly higher ($P < 0.05$) uptake of 2-NBDG in granulosa cells collected at 4h post-hCG as compared to those at 0h hCG. In addition, Slc2a1 mRNA was more abundant ($P < 0.01$) in 4h-hCG granulosa cells as compared to 0h-hCG granulosa cells. These results demonstrate that the LH-induced increase in glucose uptake in granulosa cells may be through transcriptional induction of the glucose transporter Slc2a1.

EXPRESSION OF THE ALTERNATIVE SPLICING FACTOR *ESRP1* DURING MOUSE EMBRYOGENESIS IS RESTRICTED TO SPECIFIC EPITHELIAL CELLS

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Alternative splicing increases the protein repertoire of the genome, allowing many proteins to be produced from a single gene, some isoforms even having antagonistic activities. Regulation of alternative splicing is crucial for normal embryonic development. For example, the splicing factor ESRP1 is important for maintaining the epithelial state of cells and its knockdown is implicated in epithelial to mesenchymal transitions in cancer cell lines.

We have characterized the expression of the *Esrp1* gene during early embryogenesis in the mouse using qRT-PCR and RNA *in situ* hybridizations. We show that, while expression is ubiquitous in E6.5 embryos, expression becomes localized to the definitive endoderm and chorion at E7.5. In E9.5 to E13.5 embryos, this expression becomes even more restricted to a subset of epithelial cells. Expression of this gene also correlates with the alternative splicing of several known targets such as *Cask*, *Dock7* and *Osbpl3*. From these results, we postulate that the splicing factor ESRP1 is important for the regulation of alternative splicing of epithelial-specific isoforms of proteins during development.

We are currently investigating the function of ESRP1 in EMT during pre-implantation and placental development as well as branching morphogenesis of the kidneys and lungs.

IN SEARCH OF *DE NOVO* STEROID BIOSYNTHESIS IN HUMAN PROSTATE CELL LINES AND BIOPSIES.

Monica Sakai[1,2], Daniel Martinez-Arguelles[1,2], Vassilios Papadopoulos[1,2,3,4]

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To evaluate the ability of human prostate to synthesize *de novo* steroids we examined the expression of key enzymes and proteins involved in steroid biosynthesis and metabolism. WPMY-1, WPE1-NA22, BPH-1, PC-3 and LNCaP cell lines were used as well as BPH and PCa specimens. Gene expression was detected by QPCR and steroid biosynthesis was assessed by radiometric HPLC. All cell lines express HMGCoA reductase, whereas the mitochondrial translocator protein TSPO and cholesterol side chain cleavage enzyme CYP11A1, first steps in steroidogenesis, were found only in WPMY-1, BPH-1 and LNCaP. The enzymes involved in androgen formation (3βHSD1, 3βHSD2 and CYP17) were not found in most of them. Only the normal cells were found to express CYP19A1 while all cells expressed HSD17B5, involved in estrogen and testosterone formation, respectively. The expression of the steroid metabolizing enzyme SRD5A1 was found in all cells although SRD5A2 was absent. Despite the presence of key steroidogenic proteins, WPE1-NA22 and BPH-1 cells were unable to synthesized de novo steroids from the radiolabeled precursor mevalonate. All human prostate specimens expressed TSPO, STAR, CYP11A1 and SRD5A1/2. The expression of 3βHSD1/2, CYP17, HSD17B5 and CYP19A1 in human biopsies was distinct to the profile observed in cell lines and indicated the lack of a continuous metabolic pathway. Association analysis indicated that majority of BPH (90.9%) and PCa (83.1%) contained CYP17A1, compared to Stage 0 (46.7%).These studies (i) question the use of prostate cell lines to study endogenous steroid synthesis, (ii) demonstrate that human biopsies express key steroidogenic enzymes and potentially synthesize de novo steroids, and (iii) identify CYP17A1 as a key enzyme to be used for patient stratification and treatment in BPH and castration-resistant prostate cancer. Funding: Ferring and MITACS

THE RESPONSES OF YOUNG VERSUS AGED ISOLATED GERM CELLS TO LONG-TERM *IN-VITRO* CULTURE

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Males produce germ cells continually throughout life; however, the quality of these germ cells decreases with advancing age. Aging germ cells display a reduced capacity to respond to oxidative stress. Previous studies examined developing germ cells in the presence of the supporting Sertoli cells that have antioxidant defenses to protect the germ cells. This has limited our understanding of germ cell specific responses; thus there is little information on the responses of germ cells at specific stages of spermatogenesis. This study examines the long-term *in-vitro* viability of isolated rat pachytene spermatocytes (PS) and round spermatids (RS) in the context of aging. Brown Norway rats were sacrificed at 4-mo (BNY) or 18-mo (BNA) of age, their testes decapsulated and digested, and germ cells separated using unit gravity velocity sedimentation. Fractions of PS and RS ≥83% purity were pooled, seeded onto 96-well plates, and cultured for 48-hours (5% CO_2, 32°C). Viability was assessed at various time points (T0-T48h) using trypan blue exclusion. RNA was extracted and gene expression analyzed using qRT-PCR. Viability remained constant (>87%) in PS until T21h (BNY: 89%; BNA: 87%), and in RS until T24h (BNY: 88%; BNA: 81%). Gene expression changed over-time; Sod1 levels decreased in BNA vs. BNY PS at T4h (n=5, P<0.05). Ldhc (n=5, P<0.0001) in both BNA and BNY PS were significantly reduced at T13h & 21h. These studies demonstrate that germ cells can be cultured following isolation without supporting cells and remain viable for 21- 24-hours. Reduced Sod1 in BNA PS suggests vulnerability to oxidants. Reduced Ldhc indicated activation of oxidative stress response pathways versus normal germ cell metabolism; age does not seem to affect Ldhc. This novel culture model will be an invaluable tool in studying germ cell responses. Supported by a grant from CIHR.

NEPHRIC DUCT FORMATION REQUIRES THE PRESENCE OF CLAUDIN FAMILY MEMBERS.

Annie SIMARD[1,4] , Indra R GUPTA[1,2,3,4] and Aimee K RYAN[1,2,3,4]

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Background: During kidney development, one of the first events happening is the formation of the nephric duct from the intermediate mesoderm. The duct consists of epithelial polarized cells that are linked at their apical side by tight junctions. Tight junctions regulate the passage of ions through the paracellular space and maintain apical localization of proteins. Claudin family members are important players in the composition, formation and maintenance of the tight junctions. The balance of claudin family members expressed in a cell determine the properties of the cell's tight junctions.

Hypothesis: Removal of claudins in the forming nephric duct will disrupt apico-basal polarity and cell shape therefore disrupt nephric duct formation.

Results: By using the non-toxic C-terminal portion of *Clostridium perfringens* enterotoxin (C-CPE) multiple C-CPE-sensitive claudins can be removed simultaneously from tight junctions. In the developing nephric duct, *Claudin-1*, *-3* and *-4* are expressed but only Claudin-3 and -4 are CPE-sensitive. C-CPE-soaked beads were implanted in the intermediate mesoderm on the right side of the embryo prior to nephric duct formation. Embryos were incubated until the nephric duct developed. Whole mount *in situ* hybridization analysis of *Lim1* expression, which is expressed throughout the nephric duct, revealed that C-CPE inhibited duct formation. *Osr1* expression confirmed that surrounding mesenchymal cells were not affected. I am currently investigating the effect of removal or overexpression of Claudin-1, -3 and -4 in the forming nephric duct.

Conclusion: Claudin family members are required for the epithelialization of intermediate mesoderm cells to form the nephric duct during kidney morphogenesis.

CNOT 6, AN mRNA DEADENYLASE, ACCUMULATES IN THE CORTEX OF MURINE OOCYTES, IN P BODY-LIKE RNP COMPLEXES.

Karl-Frederic VIEUX [1,2,3], Joao SUZUKI [3] and Hugh CLARKE [1,2,3]

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During growth, oocytes store large amounts of mRNA before downregulating transcription. These maternal mRNAs are kept in a quiescent state until recruited for translation in a developmentally regulated manner. This silencing is thought to be the consequence of 3' poly(A) tail deadenylation. None of the known deadenylases have been shown to be involved in mammalian oogenesis. We identified the expression of numerous deadenylases in murine oocytes, including *Cnot6*, encoding a subunit of the major cytoplasmic deadenylase CCR4-NOT. Immunoblots of oocytes and 1-cell embryos confirmed the presence of the protein and revealed a decrease in CNOT6 concentration in the oocyte at the full-grown and MII stages as well as the 1-cell embryos; coinciding with the first waves of maternal mRNA translation. We further characterized CNOT6 expression in ovarian sections and whole oocytes. We observed nuclear staining only in small oocytes. However oocytes at all stages of growth displayed cytoplasmic staining that was concentrated in foci that co-localized with the cortical actin cytoskeleton. To assess the specificity of the signal, we induced a siRNA-mediated knock-down of *Cnot6*. RT-PCR confirmed transcript depletion, and cortical fluorescence showed significant reduction in intensity. Moreover, immunofluorescence using a marker for cortical granules, which are implicated in the block to polyspermy post-fertilization, suggests that the CNOT6 foci are unrelated. Other proteins involved in mRNA processing are also cortically localized. This suggests that in murine oocytes, CNOT6 may be expressed transiently in the nucleus and stably in the cytoplasm in P body-like ribonucleoprotein complexes localized at the cortex.

PITX2C N-TERMINUS CANDIDATE INTERACTION PARTNERS REGULATE GENE EXPRESSION

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Left-right axis formation during embryogenesis is critical for proper physiological functioning. It is required to position organs asymmetrically in the limited space of the body cavity and to form asymmetric organs including the heart and lungs. One of the essential transcription factors is a bicoid-like homeodomain transcription factor, Pitx2c. Although its evolutionarily conserved role in left-right patterning is well known, the molecular mechanisms and downstream targets of Pitx2c still remain to be understood. Recently in our lab, we showed that there is a critical interaction domain at the N-terminus of Pitx2c that is important for its function in left-right axis formation. To further understand the role of the interaction domain in the N-terminus of Pitx2c in left-right patterning, we are identifying protein interaction partners of the N-terminus of Pitx2c. A yeast-two-hybrid screen was performed using mouse Pitx2c N-terminus as bait and mouse E11 cDNA library as prey to isolate candidate protein interaction partners of Pitx2cN. Candidate interaction partners were then tested for auto-reactivity and interactions were confirmed by yeast co-transformations. A total of 4.05×10^7 clones were screened. Thirty-two unique candidate protein interaction partners were identified. Fourteen candidates were identified by two or more clones and prioritized for further analysis. Of the fourteen, seven were not auto-reactive and interactions with Pitx2c N-terminus were confirmed. The majority of these seven candidate interaction partners have roles in gene regulation including chromatin remodeling and splicing. This data suggests that Pitx2c may organize left-right asymmetry by interacting with other regulators of gene expression.

BREAKTHROUGHS IN REPRODUCTION AND DEVELOPMENT

Research Day 2013

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

May 1, 2013

List of participants

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Shihadeh Anani*
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Nathalie Bédard
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Robert Berger
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Jinjiang Fan
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Shawn Fayer**
Marie-Lyne Fillion*
Ellis Fok
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Dayana Krawchuk
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Romain Lambrot*
Pavine Lefevre*
Manuela Lemoine
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Krista Zeidan
Jibin Zeng
Li Zhang
Xiao Yun Zhang
Xiangfan Zhang
Ziang Zhou

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

NOUVELLES AVANCÉES EN REPRODUCTION ET DÉVELOPPEMENT

Journée de recherche 2013

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

le mercredi 1 mai 2013

Liste des participants

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