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

BREAKTHROUGHS IN REPRODUCTION AND DEVELOPMENT

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NOUVELLES AVANCÉES EN REPRODUCTION ET DÉVELOPPEMENT

Research Day

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

Thursday, May 7, 2009

Palmer Howard Amphitheatre, 6th Floor
McIntyre Medical Building, McGill University
Montreal, Quebec

McGill

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

BREAKTHROUGHS IN REPRODUCTION AND DEVELOPMENT

Research Day Program

8:45 a.m.	Welcome
9:00 a.m.	**Norman Hecht**, University of Pennsylvania "Germ cells, transposons, and non-coding RNAs"
10:00 a.m.	**Nathalie Bédard** "Deubiquitinating enzyme USP2 is involved in spermatogenesis and required for NORMAL Male Fertility"
10:15 a.m.	**Géraldine Delbes** "PABP interacting protein 2 (Paip2) is a major translational regulator involved in the maturation of male germ cells and male fertility"
10:30 a.m.	**Liga Bennetts** "Characterisation of lipoma preferred partner during spermatogenesis"
10:45 a.m.	Health break
11:00 a.m.	**A.R. Souza** "Peroxideroxins are differentially modified by oxidative stress in human spermatozoa"
11:15 a.m.	**Ava Schlisser** "Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a target for teratogen-induced oxidative stress during organogenesis"
11:30 a.m.	**Aimée Ryan**, MUHC, Research Institute "Creating embryonic asymmetries - the role of Pitx2c"
12:00 p.m.	Lunch (provided) and Research poster viewing
2:00 p.m.	**Sarah Kimmins**, Department of Animal Science, McGill University "Epigenetic mechanisms in normal and pathological states in the testis"
2:30 p.m.	**Serge McGraw** "Deficiency in maternal DNMT1o disrupts global DNA methylation"
2:45 p.m.	**Adriana Cristina Ene** "The mechanism of oocyte loss in fetal and neonatal mouse ovaries"
3:00 p.m.	**Mahmoud Aarabi** "Microinjection of recombinant PAWP into metaphase II arrested *Xenopus Laevis* oocytes induces intracellular calcium release and oocyte activation"
3:15 p.m.	**Dan Arnold** "Transient competition as a tool for investigating the role of oocyte-stored histones on embryo development"
3:30 p.m.	Health break
3:45 p.m.	**Philippa Saunders**, University of Edinburgh "Endometrium - a dynamic multicellular tissue"
4:45 p.m.	Concluding remarks

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NOUVELLES AVANCÉES EN REPRODUCTION ET DÉVELOPPEMENT

Programme de la Journée de recherche

8 h 45	Mot de bienvenue
9 h	**Norman Hecht**, University of Pennsylvania "Germ cells, transposons, and non-coding RNAs"
10 h	**Nathalie Bédard** "Deubiquitinating enzyme USP2 is involved in spermatogenesis and required for NORMAL Male Fertility"
10 h 15	**Géraldine Delbes** "PABP interacting protein 2 (Paip2) is a major translational regulator involved in the maturation of male germ cells and male fertility"
10 h 30	**Liga Bennetts** "Characterisation of lipoma preferred partner during spermatogenesis"
10 h 45	Pause
11 h	**A.R. Souza** "Peroxideroxins are differentially modified by oxidative stress in human spermatozoa"
11 h 15	**Ava Schlisser** "Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a target for teratogen-induced oxidative stress during organogenesis"
11 h 30	**Aimée Ryan**, Institut de recherche du CUSM "Creating embryonic asymmetries - the role of Pitx2c"
12 h	Dîner (fournie) et presentation d'affiches
14 h	**Sarah Kimmins**, Department of Animal Science, McGill University "Epigenetic mechanisms in normal and pathological states in the testis"
14 h 30	**Serge McGraw** "Deficiency in maternal DNMT1o disrupts global DNA methylation"
14 h 45	**Adriana Cristina Ene** "The mechanism of oocyte loss in fetal and neonatal mouse ovaries"
15 h	**Mahmoud Aarabi** "Microinjection of recombinant PAWP into metaphase II arrested *Xenopus Laevis* oocytes induces intracellular calcium release and oocyte activation"
15 h 15	**Dan Arnold** "Transient competition as a tool for investigating the role of oocyte-stored histones on embryo development"
15 h 30	Pause
15 h 45	**Philippa Saunders**, University of Edinburgh "Endometrium - a dynamic multicellular tissue"
16 h 45	Mot de conclusion

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Invited Speakers
Présentateur et présentatrices invités

Norman B. Hecht, University of Pennsylvania (9:00 – 10:00 a.m.)
"Germ cells, transposons, and non-coding RNAs"

Aimée Ryan, MUHC, Research Institute (11:30 a.m. – 12:00 p.m.)
"Creating embryonic asymmetries - the role of Pitx2c"

Sarah Kimmins, Department of Animal Science, McGill University (2:00 – 2:30 p.m.)
"Epigenetic mechanisms in normal and pathological states in the testis"

Philippa TK Saunders, University of Edinburgh (3:45 – 4:45 p.m.)
"Endometrium - a dynamic multicellular tissue"

GERM CELLS, TRANSPOSONS, AND NON-CODING RNAS

Norman B. Hecht

Center for Research on Reproduction and Women's Health, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-6080 USA

Germ cells are full of strange transcripts that do not encode proteins. Piwi-family proteins are required for the processing and perhaps stabilization of one class of small non-coding RNA named piRNAs. piRNAs are an extremely abundant (hundreds of thousands) group of small germ cell RNAs of 26-32nt that usually start with a 5' uracil, are evolutionarily conserved but not sequence conserved, and are predominantly sense strands. They are distinct from microRNAs and believed to be derived from long precursor RNAs by a Dicer independent mechanism. piRNAs have been proposed to have many functions in the male germ line including repression of transposons, regulation of translation, and targeting regions of the genome for epigenetic change. Gene targeting of each of the murine Piwi-family proteins disrupts spermatogenesis with a strong indication that Piwi proteins and/or their piRNA partners are involved in transposon control. To gain insight into the function(s) of piRNAs in male germ cells containing normal amounts of Piwi proteins, we have gene targeted a region of chromosome 2 that encodes piRNAs. Deletion/reduction of ~500 piRNAs from this locus does not affect spermatogenesis, fertility, or the phenotype of the null mice, but a dramatic increase in the retrotransposition protein ORF 1 is seen. This up-regulation of ORF1 is complementary to and supports published reports describing the activation of transposable elements when the piRNA-binding proteins, Mili or Miwi2, are deleted. In these mutants, spermatogenic arrest also occurs, suggesting additional functions for these RNA-binding proteins or a more dramatic effect from the loss of many (most?) piRNAs. Moreover, the population of small RNAs, commonly designated piRNAs, is highly heterogeneous with a sub-population of small RNAs that selectively bind RNA-binding proteins such as MSY2. Applying a cross-linking and immunoprecipitation procedure (the CLIP assay) with an affinity-purified antibody to MSY2, we have recently discovered that the MSY2 protein selectively binds a novel population of small testicular RNAs (MSY-RNAs). MSY-RNAs are ~26-32 nucleotides, often initiate with a 5’ adenine, and are expressed throughout germ cell differentiation. MSY-RNAs are also expressed in somatic cells. Although most of the MSY-RNAs are derived from annotated genes, a small number (16 of a total of 230 clones sequenced) are piRNAs. All piRNAs are believed to be processed from long transcripts by a MIWI-dependent mechanism. To our surprise, the piRNAs that selectively bind MSY2 are expressed in mice lacking Miwi and the temporal expression of these piRNAs differs greatly from other known piRNAs. Much remains to be understood regarding non-coding RNA metabolism and functions in eukaryotic cells.

Norman B. Hecht, PhD

POSITIONS AND EMPLOYMENT:

1970-1976	Assistant Professor, Department of Biology, Tufts University
1976-1983	Associate Professor, Department of Biology, Tufts University
1978-1979	Visiting Scientist, University of Amsterdam Medical Center and University of Turku, Finland
1983-1997	Professor, Department of Biology, Tufts University
1986	Fogarty Fellow, University of Melbourne, Australia
1993-1994	Varon Visiting Professor, Weizmann Institute, Israel
1997-	William Shippen, Jr. Professor of Human Reproduction, Department of Obstetrics and Gynecology, University of Pennsylvania, Philadelphia, PA
2007	Visiting Professor, University of Melbourne
2007	Visiting Professor, University of California, Santa Cruz
2007	Visiting Professor, Stanford School of Medicine
2008	Visiting Professor, University of Rome, La Sapienza

OTHER EXPERIENCE AND PROFESSIONAL MEMBERSHIPS:

1986	Fogarty Senior Investigator Fellowship
1986, 1988	Vice-Chairman and Chairman of Gordon Conference on "Mammalian Gametogenesis and Embryogenesis"
1986-1987	Consultant to the Panel on Reproductive and Developmental Toxicology, Committee on Biological Markers, Board on Environmental Studies and Toxicology, National Research Council
1986-1990	Editorial Board, Journal of Andrology
1988-1999	Associate Editor, Molecular Reproduction and Development
1989-	Associate Editor, Journal of Cellular Biochemistry
1989-1992	Member of Steering Committee of the Task Force on Methods for the Regulation of Male Fertility, World Health Organization
1989-1992	Member of Clinical Sciences Study Section (Subcommittee 3), Division of Research Grants, NIH.
1993-1997	Member of Reproductive Biology Study Section, Division of Research Grants, N.I.H.
1998-	Member of Ad Hoc Advisory Committee of the Research Group on Methods for Male Fertility Regulation, World Health Organization
1998-2007	Associate Editor, International Journal of Andrology
1999-	Editorial Board, Biology of Reproduction
2007-	Associate Editor for Reviews, Biology of Reproduction
2008-	Editorial Board, Journal of Andrology
2008-	Editorial Board, Systems Biology in Reproductive Medicine

HONORS:

1996	Serono Award of American Society of Andrology
2000	Society for the Study of Reproduction Research Award
2006	Distinguished Andrologist Award of American Society of Andrology

CREATING EMBRYONIC ASYMMETRIES: THE ROLE OF PITX2C

Dr. Aimee Ryan

MUHC, Research Institute

The primary focus of our research is to understand the molecular and morphological events that distinguish the left and right sides of the embryo and are critical for determining how the internal organs are positioned within the body cavity. In vertebrates, the majority of the internal organs are unpaired and localized asymmetrically with respect to the midline. The asymmetric placement of organs allows for their efficient packing within the body and is required for normal physiological function. Over the past 10 years several dozen molecules that participate in determining 'leftness' and 'rightness' have been identified and placed into a patterning cascade. However, the mechanisms that convert the 'left' and 'right' information into asymmetric organ morphogenesis remain elusive.

At the downstream end of this cascade is Pitx2c, a member of the paired class of homeodomain proteins. Pitx2c is asymmetrically expressed in the left lateral plate mesoderm and on the left side of many of the organs that become asymmetrically positioned. Although Pitx2c plays an evolutionarily conserved role in left-right patterning, neither its transcriptional targets nor the molecular mechanisms through which it exerts its patterning function are known. We are exploring the role of the Pitx2c N-terminal domain in Pitx2c's left-right patterning function. Overexpression of the Pitx2c N-terminus on the left side of chick embryos randomizes the direction of heart looping, the first morphological asymmetry conserved in vertebrate embryos. We have recently defined a 5 amino acid domain encompassing Leucine-41 in the Pitx2c N-terminus that is critical for this activity. Interestingly, the Pitx2c N-terminus does not inhibit the transactivation function of Pitx2c in transient transfection studies and leucine-41 does not appear to be critical for full-length Pitx2c to activate expression of bicoid responsive or the procollagen lysyl hydroxylase (*Plod*-1) promoters. Our data suggest that protein interactions with the Pitx2c N-terminus are required to activate expression of genes that are essential for establishing left-right asymmetries. We are currently testing this hypothesis.

Aimee Ryan, PhD

BIOSKETCH:

B.Sc. Honours (Genetics), University of Manitoba	1985
M.Sc. in Biology, Queen's University (Human Genetics, Supervisor: Dr. B.N. White)	1988
Ph.D. in Biochemistry, Queen's University (Molecular Biology, Supervisor: Dr. R.G. Deeley)	1994
Postdoctoral Fellowship, Faculty of Medicine, UCSD (Supervisor: Dr. M.G. Rosenfeld)	
Assistant Professor, McGill University	2000

CURRENT RESEARCH:

I am a developmental and molecular biologist interested in understanding the molecular mechanisms underlying normal development of the vertebrate embryo. The major goal of my research is to provide a clearer understanding of the molecular and morphological events involved in formation of the left-right axis, from transcriptional events in the nucleus to changes in cell-cell interactions. Proper formation of this axis is essential to direct subsequent asymmetric development and positioning of the internal organs, which permits their efficient packing within the body cavity. The organization of the internal organs is evolutionarily conserved in vertebrates: variations from the normal pattern disrupt physiological functioning and usually lead to life threatening complications.

The goal of the research in my lab is to identify molecules that participate in formation of the left-right axis and to define the mechanisms through which they effect asymmetric morphogenesis. As with all patterning events, asymmetric morphogenesis requires the conversion of extracellular signals into changes in intracellular gene expression that lead to cell/tissue remodeling, migration and differentiation. Although several molecules have been placed into a left-right signalling cascade, many are still unknown, and precisely how they direct the morphogenetic mechanisms remains obscure. In my laboratory, we are undertaking two approaches to improve our understanding of this process.

First, we are studying the homeodomain transcription factor, Pitx2c. We showed that Pitx2c has an evolutionarily conserved role in patterning the left-right axis and that it is the most downstream component of the pathway that directs asymmetric organ development. Pitx2c is asymmetrically expressed on the left side of tissues that are important for directing asymmetric morphogenesis, and in organs that become asymmetrically positioned with respect to the midline. Despite the fact that Pitx2c is a transcription factor, no gene targets have been identified with respect to left-right patterning. Currently, in my laboratory, we are characterizing the molecular mechanism(s) employed by Pitx2c to exert its developmental effects. Our future goals include the identification of Pitx2c transcriptional targets specific to left-right patterning.

Our second approach has been to identify new molecules involved in forming the left-right axis. To do this, we performed a subtractive screen for factors that are differentially expressed on the left and right sides of the embryo. Currently, we are studying the tight junction protein, Claudin-1. Tight junctions are critical for maintaining cell polarity and determining how cell layers interact since they regulate the paracellular transport of small molecules between cell layers. We showed that overexpression of Claudin-1 on the right side of the embryo randomizes the direction of heart looping, the first gross morphological sign of left-right asymmetry. We are now investigating the role of endogenous Claudin-1 and its interactions with other proteins at the tight junction cytoplasmic plaque in this process. In addition to Claudin-1, several other interesting molecules were identified in our screen and these will be the focus of future experiments. Characterizing the molecules that regulate the function of Pitx2c and those associated with Claudin-1 function will provide missing links in the left-right patterning program and open up new frontiers in our understanding of left-right axis formation and the development of asymmetry.

We are also exploring other functions of Claudin-1 and other members of the claudin family of tight junction proteins during embryogenesis. For example, Claudin-1 expression is dramatically downregulated in the cells that leave the epiblast to ingress through the primitive streak during gastrulation and during neural tube closure; defects in these processes lead to early pregnancy loss and serious developmental defects such as spina bifida. In addition, downregulation of Claudin-1 expression in tumour cells is correlated with increased metastasis and poorer survival rates. Thus, understanding how these molecules function in normal development is likely to contribute to a better idea of the pathophysiologies involved during abnormal development and disease.

EPIGENETIC MECHANISMS IN NORMAL AND PATHOLOGICAL STATES IN THE TESTIS

Sarah Kimmins, Assistant Professor

Department of Animal Science, associate member of Department of Pharmacology and Therapeutics. McGill University, Montreal Canada

The Kimmins lab is focused on determining how lifestyle factors such as nutrition can interact with the epigenetic layer in developing germ cells to alter male reproductive health. Germ cell development is a critical period during which epigenetic patterns are established and maintained. The progression from diploid spermatogonia to haploid spermatozoa involves stage- and testis-specific gene expression, mitotic and meiotic division, and the histone-protamine transition. All engender unique epigenetic controls. Epigenetic events in the testis have just begun to be studied. Our latest work on the function of specific histone modifications, chromatin modifiers, and the impact of the folate on developing sperm suggests that the correct setting of the epigenome is required for male reproductive health. Strikingly, we have found that aberrant epigenetic profiles, in the form of anomalous histone modifications are characteristic of cancerous testis cells. Underscoring the importance of understanding how epigenetic marks are set and interpreted is evidence that abnormal epigenetic programming of gametes and embryos contributes to heritable instabilities in subsequent generations.

Sarah Kimmins, PhD

Joined McGill in Sept. 2005 following a post-doctoral fellowship in Strasbourg, France at the Institut de Génétique et de Biologie Moléculaire et Cellulaire, University of Louis Pasteur. Obtained a Ph.D. in 2003 specializing in Molecular Reproduction from Dalhousie University, Halifax Nova Scotia.

The Kimmins research group focuses on the role of the environment in control of gene expression by acting on a heritable layer of biochemical information known as the epigenome. The epigenome comprises DNA methylation and chemical modification of the DNA packaging proteins, the histones. The epigenome controls whether a gene is turned on or off, and mutations in the epigenetic layer have been implicated in a wide range of diseases including cancer, diabetes and imprinting disorders. At fertilization the paternal genome passes on key epigenetic information to the embryo and errors in this information can compromise the embryo and alter adult health. We are investigating how factors such as diet and xenobiotics can alter fertility through epigenetic mediated changes. Also under investigation is the role of epigenetics in the pluripotency of stem cells and in the pathogenesis of testis cancer. For our studies we use transgenic mouse models in combination with cell lines and chromatin molecular biology techniques to define the underlying epigenetic mechanisms governing gene expression. This research is funded by NSERC, FQRNT, CIHR and CFI.

ENDOMETRIUM: A DYNAMIC, MULTI-CELLULAR TISSUE REGULATED BY SEX STEROIDS

Philippa TK Saunders and *Hilary OD Critchley

*MRC Human Reproductive Sciences Unit, and *Division of Reproductive and Developmental Sciences, The University of Edinburgh Centre for Reproductive Biology. Edinburgh, UK.*

The human endometrium is a multi-cellular tissue that undergoes dynamic remodelling in response to sex steroids. As each ovarian cycle progresses, cells within the epithelial and stromal compartments serially proliferate and differentiate in preparation for implantation of the conceptus; in the absence of pregnancy, the upper functional layer is shed (menses). The endometrium also hosts a local population of immune cells including a phenotypically distinct group of natural killer cells (uNK) that congregate at the maternal-fetal interface and play an essential role in establishment of a successful pregnancy.

Receptors capable of binding progesterone (PR), oestrogen (ERα, ERß), androgen (AR) and glucocorticoids (GR) are all expressed within the human endometrium. We have mapped expression of these proteins in staged, full thickness endometrial biopsies (including the functional and basal zones of the endometrium and adjacent myometrium). ERα and ERß were both expressed in endometrial glands and stroma; in the functional layer immunoexpression of ERα declines during the secretory phase but although expression of ERß in the epithelial cells declines expression in the stroma remains unchanged. Notably, both endothelial and uNK cells express ERß but not ERa. Oestrogen receptor related beta (ERRß) is an orphan receptor that shares significant sequence homology with oestrogen receptors ERα and ERß. Transcripts corresponding to the long and short splice variants of ERRß were present in endometrial extracts and protein has been immunolocalised to epithelial, stromal, endothelial, uNK cells and macrophages. Some cells co-expressed ERRß and ERα or ERß. In vitro studies revealed that the long, but not the short, isoform of ERRß enhanced ERα-dependent ligand-induced gene expression activation. AR was only detected in endometrial stromal cells except in tissues obtained when progesterone levels had declined (late secretory phase) when expression was up-regulated in glandular epithelial cells.

Array analyses have been performed using RNA isolated from primary uNK cells (CD56+/CD3-/CD16^{dim}) incubated with or without oestradiol-17ß (E2) for 2 hours and endometrial stromal cells incubated with DHT for 2 and 8 hours. Using uNK cells E2-dependent changes were identified in 46 transcripts (threshold 1.5, $p<0.001$); changes in 11 were validated using qRTPCR. Pathway analysis suggested that treatment with E2 modified the cytoskeleton and functional assays confirmed increased polymerisation of F-actin. In stromal cells incubated with DHT the most notable changes in gene expression occurred at 2 hours with the majority resulting in reduced expression of target genes.

In summary, our studies using primary human endometrial tissues and cells have begun to elucidate some of the changes that occur in response to sex steroids in this tissue.

Philippa TK Saunders, PhD

Philippa Saunders has a BSc in Microbiology from Bristol University and a PhD in Reproductive Biology from Cambridge University.

She spent three years as a postdoctoral fellow at the University of Florida in Gainesville USA. After returning to the UK she undertook research fellowships at the Zoological Society in London and in the Department of Obstetrics and Gynaecology in Edinburgh.

Philippa was appointed as a Principal Investigator at the Medical Research Council Human Reproductive Sciences Unit in Edinburgh in 1989 and awarded tenure in 1993.

Since 2005 she has been an honorary Professor of the University of Edinburgh.

In May 2007 Philippa became Convenor of the Centre for Reproductive Biology.

RESEARCH FOCUS:

The primary focus of my research group is to determine how steroid hormones, such as androgens and oestrogens, influence the formation and function of the reproductive systems in men and women. We have also been investigating which molecules influence the maturation of the testicular gametes from stem cells into sperm.

BACKGROUND TO THE RESEARCH:

Steroids such as androgens and oestrogens, that are made in the testes and ovaries of men and women not only regulate our fertility but also have a major impact on our cardiovascular system, our brain, our bones and our immune system. In the adult, development of the gametes (eggs and sperm) takes place within two highly specialised environments; the follicle of the ovary and an interconnected series of tubes within the testis known as the seminiferous epithelium.

In both organs the development of the gametes (gametogenesis) is dependent upon the support from somatic cells (female somatic cells are called granulosa cells and male somatic cells are called Sertoli cells). Our quest to understand how steroids alter cell function has focused upon investigating the expression and functional activity of receptors that bind to androgens (androgen receptor, AR) or oestrogens (oestrogen receptor, ER) in reproductive organs such as the testis and endometrium and in some cancers.

Infertility affects 1 in 5 couples in the UK , of these about a third are due to problems in the male partner. In men the adult testis performs two essential functions; the synthesis and secretion of steroid hormones and the production of mature sperm from immature spermatogonial stem cells (a process known as spermatogenesis). Spermatogenesis depends upon interactions between the Sertoli cells and the germ cells.

RECENT PROGRESS:

Testis function

In collaboration with groups in Leuven (Belgium) we have been studying mice in which androgen receptor function in the testis has been altered. Mice who do not have androgen receptors in their Sertoli cells are infertile because the process of spermatogenesis is disrupted. Studies on these mice in parallel with those on cell lines are providing us with novel insights into the way(s) in which androgens support male fertility. Complementary studies have been investigating how testicular stem cells are regulated and the way(s) in which the local environment of the testis can have an impact on the DNA within the sperm.

Oestrogens and receptor activity

We have been using receptors that have a fluorescent protein tag to investigate how binding to ligands (natural and synthetic oestrogens) alters the location of different types of oestrogen receptors within cells and the impact this has on expression of target genes. In collaboration with Professor Hilary Critchley we have mapped the cell-specific patterns of expression of oestrogen receptors and oestrogen receptor related proteins in endometrium recovered from normal cycling women (Figure 1). We found that the different cell types contain different mixtures of receptors and this has made us re-think how cell function is regulated.

Oral Presentations
Présentations orales

O1 - Nathalie Bédard (10:00 a.m.)

O2 - Géraldine Delbes (10:15 a.m.)

O3 - Liga Bennetts (10:30 a.m.)

O4 - A.R. Souza (11:00 a.m.)

O5 - Ava Schlisser (11:15 a.m.)

O6 - Serge McGraw (2:30 p.m.)

O7 - Adriana Cristina Ene (2:45 p.m.)

O8 - Mahmoud Aarabi (3:00 p.m.)

O9 - Dan Arnold (3:15 p.m.)

DEUBIQUITINATING ENZYME USP2 IS INVOLVED IN SPERMATOGENESIS AND REQUIRED FOR NORMAL MALE FERTILITY

Nathalie Bedard[1], Yaoming Yang[1], Marie Plourde[1], Louis Hermo[2], Dan Cyr[3], Carlos Morales[2], Hugh Clarke[4] and Simon S. Wing[1]

[1]*Polypeptide Hormone laboratory, Department of Medicine, McGill University, Montreal, Quebec, Canada.*
[2]*Department of Anatomy and Cell Biology, McGill University, Montreal, Quebec, Canada*
[3]*INRS-Institut Armand Frappier, Université du Québec, Laval, Québec, Canada*
[4]*Department of Obstetrics & Gynecology, McGill University, Montreal, Quebec*

Spermatogenesis is a complex developmental process in which there is both large scale and selective degradation of proteins. The roles of the ubiquitin (Ub) system in this process remain to be elucidated. Previously, we found that protein ubiquitination plays an important role during spermatogenesis and characterized several enzymes involved in Ub conjugation. More recently, we have explored the role of deubiquitinating enzymes. We identified USP2, whose gene encodes two alternatively spliced isoforms, which share identical catalytic core domains, but differ in their N-terminal extensions (Lin H. et al. Mol. Cell Biol. 20: 6568-78). Both USP2 isoforms are expressed at high levels in late elongating spermatids. To further characterize the role of USP2 in spermatogenesis, we inactivated the USP2 gene in the mouse. Such mice are viable and appear grossly normal, but males are subfertile as rare litters and decreased litter size was observed despite normal vaginal plugging by the KO males. Mating of KO males with superovulated females indicated a very low yield of fertilized embryos. Although USP2 KO sperm are motile when initially isolated, there appears to be a more rapid reduction in motility over time compared to WT sperm. Histology of the testis revealed abnormalities in maturation of elongating spermatids. Our results suggest that loss of USP2 leads to a production of sperm with defective motility and consequent hypofertility. Funded by Canadian Institutes of Health Research and Fonds de la recherche en santé du Quebec.

- O2 -

PABP INTERACTING PROTEIN 2 (PAIP2) IS A MAJOR TRANSLATIONAL REGULATOR INVOLVED IN THE MATURATION OF MALE GERM CELLS AND MALE FERTILITY

Geraldine Delbes[1], Akiko Yanagiya[2], Nahum Sonenber[2] and Bernard Robaire[1]

[l]*Department of Pharmacology and Therapeutics, McGill University, Montreal, Quebec, Canada*
[2]*Department of Biochemistry, McGill University, Montreal, Quebec, Canada*
[3]*Department of Obstetrics and Gynecology, McGill University, Montreal, Quebec, Canada*

Paip2 regulates translation by promoting the dissociation of PABP (PolyA Binding Protein) from the poly(A) tails of mRNA. There are 2 homologs of Paip2, Paip2A and Paip2B, that are highly expressed in the pancreas. In the testis, Paip2A is highly expressed whereas Paip2B levels are low. Using immunohistochemistry, we have shown that Paip2A is expressed in spermatids from steps 7-8 of spermiogenesis until the sperm are released. PABP starts to be expressed in the pachytene spermatocytes at stages 5 and its expression increases gradually in the round spermatids, to finally disappear in the elongating spermatids at step 15 of spermiogenesis. To understand the role of Paip2 in spermatogenesis, we characterized a double knock-out (DKO) transgenic mouse lineage in which both Paip2A and Pai2B genes were inactivated. When compared to wt males, the DKO males had a 6% lower body weight as well as significantly lower epididymal (25% less) and prostatic weights (71 % less). No spermatozoa could be found in the cauda epididymidis and the testis sperm head count was significantly decreased by 64%. Histological examination of the testes revealed abnormalities with vacuolization in some tubules and abnormal alignment of the spermatozoa at the lumen at stages 7-8, thus suggesting that the elongated spermatids in the DKO mouse are retained and cannot be released into the lumen properly. Moreover, we have shown an abnormal expression of PABP in the single Paip2A knock-out male germ cells where PABP is still expressed in elongated spermatids at stage 8 and staining can be found in the lumen all along the epididymis. We hypothesize that Paip2 translation factors are major players in the development of male germ cells, regulating the translation of key proteins during spermiogenesis that are involved in chromatin condensation and sperm release during spermiogenesis. Supported by CIHR.

CHARACTERISATION OF LIPOMA PREFERRED PARTNER DURING SPERMATOGENESIS

Liga Bennetts[1], Barbara Hales[1], Bernard Robaire[1,2]

Department of Pharmacology and Therapeutics[1], and Obstetrics and Gynaecology[2], McGill University

The processes that underlie the profound changes to the nuclear compartment of the male germ cell during spermatogenesis remain poorly understood. In the present study, a protein previously identified as sperm nuclear matrix/perinuclear theca component, lipoma preferred partner (LPP), was investigated by immunofluorescence microscopy in order to gain an understanding of the roles of this sperm nuclear proteins during spermatogenesis. Five micron cryosections were prepared from testicular tissue, and examined by immunofluoresent labelling. Labelling of LPP revealed a ring structure located at one pole of the nuclear periphery in early spermatids. This distribution shifted progressively to a larger, more intensely labelled ring at the median axis of the nuclear periphery of later stage round spermatids. In elongating spermatids, the signal shifted to an oval structure that contained a median longitudinal axis, which localised to the nuclear periphery, and was subsequently lost at stages of more condensed chromatin structure. Stage-specific seminiferous tubule squash preparations revealed the same pattern of labelling as determined in cryosections. Dual labelling of squash preparations with 1 µg/mL *Arachis hypogaea* showed immunolocalisation of LPP at the periphery of the developing acrosome throughout spermiogenesis. These data imply that LPP may be involved in guiding the caudal descent of the acrosome during early spermiogenesis, and in anchoring the acrosome during nuclear elongation. This investigation represents the first localisation study of this germ cell perinuclear marker during mammalian spermatogenesis.

This work was supported by CIHR.

- O4 -

PEROXIREDOXINS ARE DIFFERENTIALLY MODIFIED BY OXIDATIVE STRESS IN HUMAN SPERMATOZOA

A. R. Souza[1,3] and C. O'Flaherty[1,2,3]

[1]*Department of Surgery (Urology)*
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Peroxiredoxins (PRDXs) are a recently discovered family of thiol peroxidases. They play an important role in cell peroxide and peroxynitrite detoxification and in the regulation of hydrogen peroxide (H_2O_2) signaling. The aim of this study was to characterize PRDXs in human spermatozoa challenged with oxidative stress. Sperm proteins from Triton X100-treated and non-treated sperm and from spermatozoa challenged with a mild (0.05 and 0.1 mM H_2O_2) or a strong (0.35-5 mM H_2O_2) oxidative stress were electrophoresed under non- and reducing conditions. PRDX 4, 5 and 6 were found in both Triton-soluble and -insoluble sperm fractions, while PRDX 1 was found only in the Triton-insoluble fraction. PRDX 1, 2, 5 and 6 were immunolocalized in the sperm head and in the tail. PRDX 3 was found in the post-acrosomal region and in the principal piece. PRDX 4 was highly expressed in the acrosome region. PRDX 1, 4, and 6 were found in seminal plasma. PRDX 1 and 6 were present as protein doublets in non-treated spermatozoa under non-reducing conditions. PRDX 1 and 6 were found as bands of high molecular weight (>170 kDa) in spermatozoa incubated with 0.35-5 mM H_2O_2 (strong oxidative stress). PRDX 6 became a strong single band due to 0.05 mM H_2O_2 (a condition that promotes sperm capacitation). H_2O_2 (0.35-5 mM) caused a decrease of the signal for PRDX 4 and 5 compared to non-treated cells. In conclusion, PRDXs are differentially modified by oxidative stress, suggesting a role of PRDXs as antioxidants and as potential modulators of H_2O_2 action in human spermatozoa.

**GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE (GAPDH) AS A TARGET FOR
TERATOGEN-INDUCED OXIDATIVE STRESS DURING ORGANOGENESIS**

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New insights into an evolutionarily conserved glycolytic enzyme, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), have revealed that it plays important roles during oxidative stress. Nuclear translocation of GAPDH following oxidative stress directly reactivates oxidized proteins involved in DNA repair, such as APE1, and stimulates catalytic activity of p300/CBP, that activates p53 to initiate apoptosis. Oxidative stress-induced reactive oxygen species produce lipid peroxidation end-products such as 4-hydroxynonenal (4-HNE), a reactive electrophile that forms protein adducts. *In utero* exposure to hydroxyurea (HU), a model teratogen, GAPDH-4-HNE conjugates are formed in the caudal region of organogenesis stage embryos. The formation of GAPDH-4-HNE protein adducts alters GAPDH enzymatic activity and induces its nuclear translocation. To test this hypothesis, CD-1 mice received saline or HU (400 or 600 mg/kg) on GD9; embryos were excised 3 hrs later and separated into head, mid-body, and tail for enzyme assays or fixed and sectioned for GAPDH immunofluorescence, and caspase-3 activation. In control embryos, GAPDH activity was similar along the anterior to osterior axis (~10 units NADH/mg protein/min). Low dose HU decreased GAPDH activity only in the head region (~7.5 units, $p<0.05$). High dose HU decreased GAPDH activity only in the body region (~6.5 units, $p<0.05$). Interestingly, GAPDH was cytoplasmic throughout the control embryos; however, HU induced a dose-dependent increase in the nuclear localization of GAPDH reactivity in the neural tube and lumbar sacral somites of the caudal region. There was also an increase in pyknotic nuclei coinciding with caspase-3 activation in the caudal region. The nuclear translocation of GAPDH may be involved in initiating a p53-dependent cell death cascade. Supported by CIHR.

- O6 -

DEFICIENCY IN MATERNAL DNMT1O DISRUPTS GLOBAL DNA METHYLATION

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DNA methylation is an epigenetic mark catalyzed by DNA cytosine methyltransferase (DNMT) enzymes and is involved in a variety of developmental processes. Mice embryos born from oocyte-derived DNMT1o deficient mothers (*Dnmt1o* mat-/-) have disrupted genomic imprinting and display significant anatomical abnormalities. Here, we reveal that placentas from 9.5 dpc *Dnmt1o* mat-/- conceptuses have a much higher incidence of abnormalities compared to males. Using thin layer chromatography (TLC), we did not uncover alteration of embryonic DNA methylation in *Dnmt1o*-deficient 9.5 dpc embryos; however, female placentas displayed reduced levels of global DNA methylation compared to their male counterparts, which were mostly normal. Further analysis revealed that the repetitive DNA elements (IAP repeat and minor satellite) demonstrate selective hypomethylation in female *Dnmt1o* mat-/- placentas. By means of a more sensitive method, restriction landmark genomic scanning (RLGS), we investigated the methylation status of approximately 2000 single-copy loci and found that *Dnmt1o* mat-/- 9.5 dpc embryos and placentas display a range of aberrations (less than 5 to more than 60 loci) compared to controls. Individual embryos and placentas of both sexes predominantly exhibited hypomethylation; however, hypermethylation events were also observed. Interestingly, almost all sites that were consistently hypomethylated in XX versus XY *Dnmt1o* mat-/- placentas are X-linked CpG island-containing loci. Together these studies show that a lack of DNMT1o activity in the 8-cell preimplantation embryo stage results in wide-spread DNA methylation defects in extraembryonic tissues. Methylation defects on the X chromosome may provide a clue to the underlying nature of the sex-specific bias in placental morphology and global methylation.

THE MECHANISM OF OOCYTE LOSS IN FETAL AND NEONATAL MOUSE OVARIES

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Introduction. More than half of the maximum number of germ cells is eliminated from ovaries by neonatal life, thus limiting the oocyte reserve for reproduction. The cause or mechanism of this female germ cell loss remains largely unknown. A major loss occurs in the oocytes which reach the pachytene stage of meiotic prophase, suggesting that oocytes with meiotic or recombination errors may be eliminated by a checkpoint mechanism. The purpose of our study is to determine whether oocytes are eliminated by apoptosis and if so in which pathway. We used a *Msh5* null mutant mouse strain, in which all oocytes are eliminated by neonatal life. Msh5 encodes a protein required for meiotic chromosome synapsis.

Results. No normal pachytene oocytes were seen in the null mutant ovary at any developmental stage and a significantly loss of oocytes was found in the mutant ovary at 19.5 dpc. Caspase 9 activation increased considerably from 17.5 to 18.5 dpc and leveled at 19.5 dpc, to similar extents between the wild-type and null mutant ovaries. Caspase 2L activation also increased in a similar pattern but at much lower levels. Caspase 7 appears to be a candidate as an effector caspase.

Conclusion. Mitochondrial pathway of apoptosis appears to be a mechanism for oocyte elimination in both normal and *Msh5* null mutant ovaries.

- O8 -

MICROINJECTION OF RECOMBINANT PAWP INTO METAPHASE II ARRESTED *XENOPUS LAEVIS* OOCYTES INDUCES INTRACELLULAR CALCIUM RELEASE AND OOCYTE ACTIVATION

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We previously reported PAWP, post acrosomal sheath WW domain binding protein, as a candidate sperm borne, oocyte-activating factor. PAWP enters oocyte during fertilization and induces oocyte activation events including meiotic resumption, pronuclear formation and egg cleavage. The N-terminal of PAWP shares a high homology to WW2 domain binding protein while the C-terminal half contains a functional PPXY motif. In order to provide proof that PAWP is the primary initiator of zygotic development it is imperative to show that PAWP initiates intracellular calcium signalling, which is considered essential for oocyte activation. Utilizing *Xenopus laevis* as our model, we injected recombinant PAWP or *Xenopus* sperm into metaphase II arrested oocytes and observed a significant rise in intracellular calcium levels over controls. Concurring intensities and durations of PAWP and sperm-induced calcium waves, detected by infra-red two-photon laser scanning fluorescence microscopy, were prevented by coinjection of a competitive PPGY-containing peptide derived from PAWP but not by the pointmutated form of this peptide. This study also correlates PAWP and sperm induced calcium efflux with meiotic resumption in *Xenopus*. The similar mode of oocyte activation by sperm and PAWP provides evidence for the first time that sperm anchored PAWP is a primary initiator of zygotic development (Supported by CIHR).

TRANSIENT COMPETITION AS A TOOL FOR INVESTIGATING THE ROLE OF OOCYTE-STORED HISTONES ON EMBRYO DEVELOPMENT

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Factors stored in the mature oocyte must sustain embryo development until activation of the embryonic genome. Histone mRNAs are highly abundant in mature oocytes and are required for subsequent embryo development. The new histone proteins must acquire particular modifications prior to activation of the embryonic genome. To date studies have relied primarily on immunolocalization of histone modifications during the early stages of development, since transgenic mice models are impractical due the high number of histone genes. However, little is known on which particular modifications are critical for successful development. To address this issue, we developed a technique where in vitro synthesized histone mRNA injected into 1cell mouse embryos transiently competes with oocyte-stored histone mRNA. The coding region of histone H3f gene was inserted into pcDNA vector with EGFP linked to the 3' end. Fertilized zygotes were micro-injected with 1-2 pg of in vitro synthesized H3+EGFP mRNA and either allowed to develop or collected at specific time points. No difference in development to the blastocyst stage was detected between treated and non-treated embryos (62.3 and 65.7%, respectively). EGFP protein was detected within 1 hr of injection and was localized to the nucleus. Nuclear localization of EGFP protein was detected in 2-cell, 4-cell, 8-cell and early morula stage embryos. Expression of EGFP mRNA was detected in 4-cell embryos, suggesting the injected mRNA was not degraded prior to embryonic activation. These results suggest that micro-injection of exogenous mRNA might be a tool for detecting oocyte-stored factors that are critical for successful embryo development.

Poster Session
Séance d'affichage

P1 - Amani Batarseh

P2 - Michelle Collins

P3 - Evemie Dubé

P4 - Marvin Ferrer

P5 - Andrew Fox

P6 - Maren Godmann

P7 - Amélie Gravel

P8 - Lisanne Grenier

P9 - Mahsa Hamzeh

P10 - Steven Jones

P11 - Romain Lambrot

P12 - Sophie-Anne Lamour

P13 - Yuefei Lou

P14 - Enas Mahrous

P15 - Gurpreet Manku

P16 - Ludovic Marcon

P17 - Craig Park

P18 - Hilma Rodrigues

P19 - Didem Sarikaya

P20 - Agathe Streiff

P21 - Patrick Turmel

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PROXIMAL GC-RICH MOTIFS REGULATE CONSTITUTIVE EXPRESSION OF THE HUMAN TRANSLOCATOR PROTEIN (18 KDA) GENE IN BREAST CANCER CELL LINES

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The translocator protein (18 kDa) (TSPO), previously known as the peripheral benzodiazepine receptor, is a mitochondrial cholesterol-binding protein that has been implicated in several functions, including steroidogenesis, proliferation, apoptosis, and differentiation. Expression of the human *Tspo* gene is elevated in several cancers in a manner correlating with the increasing malignant phenotype of the tissue. In this study, the promoter directing expression of the human *Tspo* gene in hormone-dependent, non-aggressive, poor-in-TSPO, MCF-7 and hormone-independent, aggressive, and metastatic, rich-in-TSPO, MDA-MB-231 breast cancer cells has been identified, cloned and functionally characterized. Transcription initiated at multiple sites located downstream of a GC-rich promoter that lacks functional TATA and CCAAT boxes. Deletion analysis indicated that the region between -121 to +66, which contains five putative Sp1-binding sites, was sufficient to induce reporter activity up to 24-fold in MCF-7 and nearly 120-fold in MDA-MB-231 cells. At least two GC boxes within the proximal promoter were necessary for maintaining basal levels of activity. Electrophoretic mobility shift assays (EMSA) and supershift analyses indicated that Sp1 and Sp3 formed specific protein-DNA complexes with each GC-rich motif. Expression of either Sp1 or Sp3 in Drosophila SL2 cells was sufficient to increase the activity of a proximal promoter-luciferase reporter construct and no evidence of repression was observed when both Sp1 and Sp3 were co-expressed. On the other hand, overexpression of Sp1 and Sp3 in MCF-7 and MDA-MB-231 cells indicated a differential regulation of the *Tspo* promoter activity indicating that a certain balance in the levels of the different isoforms is essential for maintaining promoter activity. Knocking down Sp1 and Sp3 expression, using specific siRNAs, indicated that Sp1 is more important than Sp3 in the regulation of *Tspo* promoter activity and mRNA expression levels in MCF-7 cells. However, both Sp1 and Sp3 are required for *Tspo* promoter activity in MDA-MB-231 cells. Employment of the bisulfite modification reaction to determine the methylation status of cytosine residues revealed that the proximal *Tspo* promoter is methylated to a higher extent in MCF-7 compared to MDA-MB-231 cells. Since methylation compacts the chromatin and is known as a silencing modification, this finding may provide an explanation for the higher level of TSPO expression in MDA-MB-231 compared to MCF-7 cells. Taken together these data constitute the first functional analysis of the human *Tspo* gene promoter, which contains multiple GC boxes, and reveals that it is prone to methylation and has potential binding sites for Sp/KLF factors, at least two of which appear to be necessary for full constitutive activity in breast cancer cells.

- P2 -

THE CLAUDIN FAMILY OF TIGHT JUNCTION PROTEINS: THEIR EXPRESSION AND REGULATION DURING GASTRULATION

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During gastrulation epiblast cells undergo epithelial to mesenchymal transition (EMT) as they delaminate and ingress through the primitive streak to form the definitive endoderm and mesoderm. The dissociation of junctional complexes is essential for EMT to occur. We are examining the expression and regulation of the claudin family of integral tight junction proteins during chick gastrulation. Tight junctions are localized to the apical pole of epithelial cells and the claudin component of the tight junction is responsible for determining the ion and size selectivity of the junction. We hypothesize that claudin expression must be repressed in the epiblast cells that ingress through the primitive streak. More than 20 members of the claudin family have been identified in vertebrates. We found that *claudins -1, -3, -10, -11, -12, -22* and *-24* showed distinct expression patterns during gastrulation and we are now determining how these expression patterns are regulated. For example, c*laudin-1* is highly expressed in the epiblast layer reduced in the cells that delaminate and ingress through the primitive streak and absent from the nascent mesoderm. The c*laudin-1* promoter contains an evolutionarily conserved E-box regulatory element that is recognized by members of the Snail family of zinc-finger transcriptional repressors that play a key role in EMT. We showed that the chick *claudin-1* promoter is repressed by Snail2 *in vitro*, and that this interaction occurs through binding to the E-box element. We are currently investigating the role of Snail-2 in regulating *claudin-1* expression *in vivo*.

CLAUDINS 1, 3, 4 AND 7 ARE ESSENTIAL IN MAINTAINING BARRIER FUNCTION OF HUMAN EPIDIDYMAL TIGHT JUNCTIONS

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Transit through the epididymis is important for spermatozoa to acquire motility and the ability to fertilize an oocyte. The microenvironment of the epididymal lumen is essential for spermatozoal maturation and is created in part by the blood-epididymal barrier that is formed by tight junctions (TJ) between the principal cells that line the lumen. Human epididymal TJs are comprised of multiple claudins (CLDNs). The role of each CLDN within the epididymal junctional complex is still unknown. Using novel epididymal cell lines derived from fertile (FHCE1) and infertile (IHCE1) patients, our objectives were to determine the signalling pathways implicated in TJ formation and to assess the role of individual CLDNs in maintaining the integrity of the epididymal TJs. TJ formation was induced between cells of the FHCE1 but not those of IHCE1 as determined by measuring the transepithelial resistance (TER). This is consistent with the fact that IHCE1 cells expressed lower levels of genes encoding junctional proteins (CDH1, CDH2, CLDN1, 3, 4, 7, 8). Gene expression profiles between FHCE1 and IHCE1 cells were compared using microarrays and revealed that several genes that are known to be implicated in TJ formation were expressed at higher levels in FHCE1 cells. Furthermore, using an siRNA approach, our results indicate that TER was lost when CLDN1, 3, 4 or 7 expression was decreased. These results suggest that these individual CLDNs are essential for barrier function in human epididymal TJs and that the regulation of epididymal TJs depends on the activation of specific signalling pathways. Supported by CIHR and FRSQ.

- P4 -

CRES ISOFORMS ARE FOUND ON THE SURFACE AND IN THE OUTER DENSE FIBERS OF EPIDIDYMAL SPERMATOZOA

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The cystatin-related epididymal spermatogenic protein (CRES) is expressed both in the testis and epididymis and found associated with spermatozoa. It appears as non-gycosylated and glycosylated isoforms . The role of CRES is enigmatic and dependent on localization of its isoforms, which is the objective of this study. Our approach was to selectively fractionate epididymal spermatozoa and find by immunoblotting which sperm fractions contain CRES isoforms. The testicular origins of these isoforms were then investigated by immunohistochemical and immunogold cytochemistry. Western blots of non-ionic detergent extracts of caput sperm with anti-CRES antibody revealed two immunoreactive bands of 14- and 19-kDa while western blots of similarly treated cauda sperm showed no such bands. However, SDS-resistant isolated tails of epididymal sperm revealed a 14 kDa immunoreactive band while isolated heads contained no immunoreative bands. Testicular homogenates, on the other hand revealed two immunoreative bands of 14- and 19-kDa which were missing in CRES knockout mice. Immunohistochemical analysis of mouse spermatogenesis showed that CRES was expressed in the tail cytoplasm of elongating spermatids from steps 9-16, with a pattern reminiscent to outer dense fiber (ODF) proteins. Ultrastructural immunocytochemistry revealed that the immunogold label was concentrated over growing ODFs. We conclude that CRES resides in detergent soluble and insoluble fractions of epididymal spermatozoa. CRES isoforms in the soluble fraction are most likely secreted by elongated spermatids and/or principal cells of the caput and associate with the surface of the spermatozoa, only to disappear from the sperm surface in the cauda. The CRES isoform in the insoluble fraction is most likely translated on polyribosomes in the elongated spermatid cytosol and then assembled as part of the ODFs of the sperm tail. (Supported by NSERC and CIHR)

EXPRESSION OF PATCHED 1, SMOOTHENED AND DESSERT HEDGEHOG IN MOUSE AND RAT TESTES

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The testis is composed by cells from different embryological origins. While the Sertoli cells originate from the mesonephros, the germ cells originate from the endoderm of the yolk sac and migrate to the gonadal primordial. Postnatally, the seminiferous epithelium is considered a tissue in constant development since the gonocytes differentiate into stem cell spermatogonia which proliferate and originate differentiating spermatogonia, primary and secondary spermatocytes and spermatids. All these cells at different steps of development are organized into specific cellular associations termed stages of the cycle of the seminiferous epithelium. It is well known that disruptions and mutations of genes encoding intracellular factors, lead to partial or complete disruption of spermatogenesis. The testis is also the site of numerous neoplasia, particularly during childhood and adolescence. The hedgehog family of cell signalling molecules and associated downstream proteins play an important function in mediating tissue interactions during development and organogenesis. Patched 1 is the receptor for the hedgehog proteins. In the absence of hedgehog binding, patched 1 represses the seven-transmembrane pathway effector, smoothened. Surprisingly, very little is known on the expression of these signalling molecules in the testis. In this investigation we examined the immunocytochemical expression of patched 1, desert hedgehog and smoothened during spermatogenesis in rat and mouse testes. Our results indicate that patched 1 and smoothened are expressed in meiotic and post-meiotic cells and suggest that they play an important role during spermatogenesis via the action of dessert hedgehog produced by Sertoli cells. Supported by CIHR.

- P6 -

EXPOSURE OF GC-1 SPERMATOGONIAL-LIKE CELLS TO TRANYLCYPROMINE AND TRICHOSTATIN A ALTERS HISTONE H3 METHYLATION AND ACETYLATION AND STIMULATES THE EXPRESSION OF *GFRA1* AND *OCT4* STEM CELL MARKERS

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OBJECTIVES: Epigenetic gene regulation has been implicated in the maintenance of pluripotency in mouse embryonic stem cells. Less is known about how epigenetic mechanisms contribute to male germ cell differentiation. Previously we identified a dynamic distribution of the lysine-specific demethylase AOF2 and its target, histone H3 methylation, during spermatogenesis. In the testis, AOF2 and the histone deacetylase 1 (HDAC1) associate in a transcriptional repressor complex. We hypothesized that AOF2 and HDAC1 are critical for spermatogonial cell differentiation. The aim of this study was to determine how AOF2 and HDAC1 regulate histone H3 methylation and acetylation, and consequently gene expression in GC-1 cells.
METHODS: GC-1 cells were cultured in the presence of epigenetic modifying drugs, tranylcypromine, (inhibits AOF2), and/or trichostatin A (TSA, inhibits HDACs). The effects of the inhibitors on histone modifications were measured by Western blot. Histone modification profiles in *Gfra1* and *Oct4* promoters were analyzed by ChIP-qPCR. DNA methylation status of promoters was checked by pyrosequencing.
RESULTS: Exposure to TSA and/or tranylcypromine increased global levels of histone H3 acetylation and H3K4 mono- and dimethylation. Treatment induced the expression of spermatogonial stem cell markers *Oct4* and *Gfra1*. Chromatin immunoprecipitation analyses associated this induction with a gain in gene activating histone H3K4 dimethylation, pan-H3-, and H3K9 acetylation in *Oct4* and *Gfra1* promoters. Pyrosequencing revealed a heavily methylated *Oct4* promoter in GC-1 cells and treatment resulted in a very slight reduction in DNA methylation. The DNA methylation status of the *Gfra1* promoter was low and not affected by treatment.
CONCLUSIONS: Histone methylation and acetylation may serve in the regulation of key genes involved in spermatogonial cell differentiation.

This research is funded by NSERC and CIHR.

UNDERSTANDING THE ROLE OF THE EPIDERMAL GROWTH FACTOR IN THE REGULATION OF EPIDIDYMAL TIGHT JUNCTIONS.

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The epididymis plays a central role in the maturation, protection and storage of spermatozoa. These functions are dependent on the blood-epididymal barrier that is formed by tight junctions between epithelial principal cells. Epidermal growth factor (EGF) is expressed in the testis and is thought to be involved in spermatogenesis and is known to activate the MAPK signalling pathways. MAPKs along with other signalling pathways, such as the PI3K/Akt, pathway, have been implicated in the regulation of intercellular junctions. Testicular factors are known to play an important role in the regulation of cell-cell interactions in the epididymis. To assess whether or not testicular factors regulate the expression of kinases involved in intracellular signalling in the epididymis, proteomic analyses of kinase expression was done on intact control and orchidectomized adult rats. Quantitative immunoblots revealed the regulation of numerous protein kinases, including several MAPKs, and downstream targets of PI3K/Akt pathway by testicular factors. Using a rat epididymal cell line (RCE), we showed that EGF rapidly activated (within 5min) its receptor (EGFR) and transiently phosphorylated both Akt and $Erk_{1/2}$. Treatment with specific inhibitors of PI3K and MEK1 indicated that the activation of Akt and $Erk_{1/2}$ was mediated by two separate pathways activated by the phosphorylation of EGFR. These results indicate that the EGF signalling pathways are activated in the epididymis. The relationship between the activation of these pathways and the regulation of specific components of the blood-epididymal barrier remains to be demonstrated. Supported by CIHR and FQRNT.

- P8 -

THE EFFECTS OF PATERNAL EXPOSURE TO CYCLOPHOSPHAMIDE ON THE RATE OF SPERMATOZOAL DECONDENSATION AND THE PATTERNING OF CHROMATIN REMODELING IN EARLY POST-FERTILIZATION ZYGOTES

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Following fertilization, the spermatozoon starts decondensing into four stages: condensed, type *a*, *b* and *c* nuclei. Chromatin remodeling is then initiated, where sperm specific proteins are replaced with maternal histones. Cyclophosphamide (CPA), an anticancer and immunosuppressive agent that causes DNA crosslinks and strand breaks, is studied as a model male mediated developmental toxicant. We hypothesize that paternal exposure to CPA during spermiogenesis leads to improper packaging of the male genome, disturbing chromatin decondensation and remodelling in early zygotes. Our objectives were first to determine when, during sperm decondensation, post-translational modified histones are present on the paternal chromatin and second to assess whether CPA treatment alters the time required for sperm decondensation or the patterning of chromatin remodeling. Male Sprague Dawley rats were gavage with either saline water or CPA, 6 mg/kg/day for 4 weeks, targeting highly sensitive post meiotic male germ cells. Control and CPA treated male Sprague Dawley rats were mated overnight; early zygotes were collected the following morning and prepared for immunofluorescence analysis. Sperm decondensation occurred first at the base, then the tip and finally in the center region of the sperm head. In zygotes sired by CPA-exposed males, the rate of decondensation was accelerated significantly in type *c* sperm nuclei. We observed two distinct patterns of chromatin remodeling; H4ack12, a marker for open chromatin structure, had homogenous staining, whereas H3S10ph, a marker for condensed chromatin structure, displayed a ring-like staining around the sperm head. Chromatin remodeling varied significantly along the longitudinal section of spermatozoa within and between sperm decondensation stages but was not affected by paternal exposure to CPA. Thus, paternal exposure to CPA disturbs the compaction of chromatin structure of male germ cells, affecting the rate of sperm decondensation, without altering the distribution pattern of modified histones in early post-fertilized zygotes. Supported by CIHR.

DIHYDROTESTOSTERONE (DHT) ACTIVATES MITOGEN-ACTIVATED PROTEIN KINASE VIA ANDROGEN RECEPTOR IN MOUSE EPIDIDYMAL CELL LINES

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Androgens are the primary regulators of epididymal structure and functions. Principal cells are the major cell type of this tissue and are particularly sensitive to androgen removal. Our previous studies have identified the initial gene families and pathways activated by DHT, the 5α-reduced metabolites of testosterone, in the regressed rat epididymis at early time points. Beside the genomic action of DHT, we hypothesize that DHT causes a rapid response in epididymal cells by activation of signaling pathways through the androgen receptor. Therefore, our objective is to identify the potential signaling pathway activated by DHT in the mouse proximal caput epididymidis-1(PC-1) cell line. This cell line is grown in medium supplemented with 10% fetal bovine serum (FBS). Two passages before the experimental procedure, FBS is replaced by charcoal-filtered FBS to remove endogenous steroids. The cells (16 groups, N=5) are then subjected to different DHT concentrations (0.1nM, 1nM, and 10nM). The control groups are treated with vehicle (EtOH). Four of these groups, one for each DHT concentration, are pretreated for 30 min with the androgen receptor antagonist hydroxyflutamide (HF). Protein is extracted 10 min, 30 min, and 45 min after treatment. Western blot analysis shows that DHT activates Erk pathway at 10 min compared to control, but this activation is not sustained at 30 min and 45 min. There is no significant difference for Akt activation compared to control at any time point. Interestingly, Erk activation is blocked by HF. Therefore DHT induces rapid action and activates Erk at 10 min via an androgen receptor mediated mechanism in PC-1 cell lines. We postulate that this rapid action of androgen may ultimately act to modulate transcriptional activity of genes regulated by AR in the nucleus. The elucidation of non-classical pathway of testosterone action in the epididymis could provide new targets for the control of male fertility.

- P10 -

ABCB1 ORTHOLOG LOCALIZATION AND INDUCTION IN THE RAT EPIDIDYMIS

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The blood-epididymal barrier provides a specialized, protected microenvironment where spermatozoa transit and mature to acquire both motility and the ability to fertilize. The barrier is comprised of both tight-junctions and numerous specialized transporters that regulate the type and quantity of compounds entering the epididymal lumen. ABCB1A and ABCB1B are broad specificity ATP-binding cassette (ABC) efflux proteins capable of regulating the disposition of xenobiotics in normal tissues serving a barrier or excretory function. The objective of this study was to characterize the expression profile and inducibility of ABCB1A and ABCB1B in the adult rat epididymis. ABCB1A mRNA levels, as determined by real-time PCR, were significantly higher in the cauda regions of the epididymis when compared to initial segment, caput and corpus. ABCB1B mRNA levels were similar throughout the epididymis. Immunohistochemistry using an antisera against both forms of ABCB1 revealed a gradient distribution of the protein along the epididymis. Minimal immunostaining was observed in the epithelial cells or spermatozoa in the caput epididymidis, and progressively increased in the corpus and cauda. Interestingly, the immunoreaction in spermatozoa was detected in the lumen of the distal caput, corpus and cauda. To assess whether or not the system was inducible by environmental contaminants, cells from an immortalized epididymal cell line (RCE) were exposed to different concentrations of nonylphenol (NP), an industrial surfactant. RCE cells exposed to 20 uM NP revealed a significant induction of both ABCB1A and ABCB1B mRNA and ABCB1 total protein, suggesting that ABC efflux transporters are inducible in the epididymis. The unique expression profile and induction of ABCB1A and ABCB1B in the epididymis suggests an important role for these proteins in regulating barrier function in the male reproductive tract. Supported by Environment Canada, CIHR and the Armand-Frappier Foundation.

HISTONE METHYLATION AND DEREGULATION OF GENE EXPRESSION IN TESTICULAR CANCER

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Testis cancer is the most common form of cancer in men between the ages of 15-35. Large scale array analysis has identified genes with abnormal expression in testis cancer, but the role of histone modifications in this gene deregulation is not known. Thus, the aim of our study was to determine if changes in histone modifications play a key role in the pathogenesis of non-seminomatous tumors.

Two cell lines representative of non-seminoma, TERA-2 and NCCIT were treated with 5-Aza-2'-deoxycytidine (AZA), an epigenetic targeting drug. The impact of treatment on the expression of key genes implicated in cancer pathology was measured and compared to the histone methylation landscape at promoters. The genes studied were *OCT4*, a pluripotency marker and *RASSF1A* a tumor suppressor gene. We examined the levels of histone H3-K4 methylation which are commonly associated with active chromatin and H3-K9 methylation which are associated with repression of transcription. By RT-PCR, we determined that AZA treatment decreased *OCT4* expression and restored *RASSF1A* expression. By ChIP-qPCR, we revealed that AZA treatment decreased gene silencing H3-K9 methylation at the *RASSF1A* promoter whereas gene activating H3-K4 di-methylation was decreased but not H3-K4 tri-methylation. On the *OCT4* promoter H3-K9 mono and tri-methylation were not modified, whereas H3-K4 di-and tri-methylation were reduced. These results suggest that *RASSF1A* gene expression is re-activated following AZA treatment by a loss of gene silencing H3-K9 modifications and the maintenance of gene activating H3-K4 tri-methylation. Conversely *OCT4* gene expression is lost as a consequence of reduced gene activating H3-K4 methylation and the maintenance of H3-K9 gene silencing modifications. Thus, treatment with the epigenetic modifying drug AZA changes the chromatin landscape to restore 'normal' gene expression by altering H3-K4 and H3-K9 methylation.

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SURVIVIN, AN INHIBITOR OF APOPTOSIS PROTEIN, IS TRANSCRIPTIONALLY-REPRESSED BY TESTOSTERONE IN THE EPIDIDYMIS

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The epididymis is responsible for the proper maturation, transport and storage of spermatozoa; it is functionally divided into four regions (initial segment, IS; caput, Ca; corpus, Co; and cauda, Cd). Despite its dependence on androgens, androgen withdrawal causes little apoptosis. Previous gene expression analysis has identified survivin (Birc5), an inhibitor of apoptosis protein, as a gene potentially regulated by testosterone. Our aim was to investigate the effects of androgen withdrawal and/or maintenance on survivin expression along the epididymis. To do this, we took *in vivo* and *in vitro* approaches. *In vivo*, nine groups (n=5/group) of male BN rats were sham-operated (SO) or orchidectomized (ORC) and treated with either empty or testosterone (T)-filled implants designed to maintain control serum T concentrations. Rats were euthanized at 0.5, 1, 3, and 7 days after orchidectomy and epididymides were separated into IS, Ca, Co, and Cd for RNA extraction. For the *in vitro* studies, we used the androgen-dependent immortalized mouse epididymal PC-1 cell line. PC-1 cells were treated with charcoal-stripped (CS) serum or CS serum supplemented with 5α-dihydrotestosterone (5α-DHT) for 1 to 6 days. RNA was extracted and cell survival was assessed. In the absence of T, Birc5 mRNA expression was increased over time. T maintenance significantly repressed Birc5 increase in all regions. Similarly, in the PC-1 cell line where CS treatment did not affect cell survival, Birc5 mRNA was increased in the CS group as compared to the 5α-DHT group at all time points. Analysis of the 3kb upstream promoter of the rat Birc5 gene identified 5 potential androgen response elements (AREs) in its sequence. This study suggests that Birc5 is repressed by T at the transcriptional level and that the PC-1 cell line is a good model to study the transcriptional regulation of Birc5 in the epididymis. This work provides a better understanding of the regulation by androgens in mediating cell survival in the epididymis. Supported by CIHR.

GLOBAL GENE EXPRESSION ANALYSIS TO IDENTIFY ESTROGEN REGULATED GENES IN PREIMPLANTATION EMBRYOS

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Implantation is a process by which the blastocyst makes the first physiological contact with the maternal uterine luminal epithelium. Molecular "cross-talk" between the blastocyst and uterus is crucial for successful implantation. Ovarian steroid hormones have been shown to render the uterus receptive to the embryo. There is increasing evidence that ovarian steroids also play a role in rendering the embryo competent for implantation however, the mechanism(s) by which estrogen initiates this response still remains elusive. To identify genes whose expression is modulated by estrogen within the embryo, we examined the global gene profiles of preimplantation embryos (dpc3.5) from normal mice after PMSG and hCG superovulation, ICI 182,780 (a specific ER antagonist) treated mice after 18h hCG injection, and of embryos recovered from ligated oviducts. Surprisingly, only 25 genes were down-regulated by 1.5 fold in the embryos from ICI treated uterus compared with those from normal uterus. Of these genes, 10 were also shown to be down-regulated in the embryos from ligated oviducts. 53 genes were up-regulated by at least 1.5 fold in the embryos from ICI treated uterus compared with those in normal uterus. Of these 53 genes, 27 genes were up-regulated only in ICI treated samples but not in embryos from normal uterus or ligated oviducts.

Amongst the genes identified as down-regulated by ICI or ligation were ErbB2 (EGF-receptor, EGF-R) and Psg23 (Pregnancy specific gene) whereas Btg1 (B cell translocation gene 1), an inhibitor of G0/G1 transition, and Adh1 (alcohol dehydrogenase 1) were found to be up-regulated. Our results demonstrate that ovarian estrogen not only modulates target genes in the uterus to render it receptive but also modulates expression of genes required for the implantation competence of the embryo. The identification of unique genetic markers in pre-implantation embryos for the onset of implantation helps to define additional aspects of the embryo-uterine cross-talk that underlies the implantation process.

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QUANTITATIVE ASSESSMENT OF MITOCHNDRIAL DNA COPY NUMBER IN MOUSE OOCYTES

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Mitochondria play a primary role in a variety of cellular functions including Ca^{2+} homeostasis, production of reactive oxygen species, and regulation of intracellular metabolites. Growing oocytes accumulate a large number of mitochondria. These are required to support early embryonic development as mitochondrial replication in embryos does not begin until after the blastocyst stage. Low mitochondrial content in oocytes has been linked to fertilization failure, ovarian insufficiency and poor oocyte quality; however, a wide range in the number of mitochondria per oocyte has been reported. We propose that growing oocytes may have to accumulate a certain 'threshold' number of mitochondria in order to be able to develop as embryos. To test this, we are developing a PCR-based assay to measure mitochondrial DNA (mtDNA) content of individual oocytes and embryos. We identified primers that efficiently amplify mtDNA sequences. To obtain a reliable estimate of mitochondrial DNA (mtDNA) content, we plan to take into account the efficiency of DNA recovery from single oocytes. This was determined by measuring the amount of rabbit globin cDNA – an exogenous DNA added to the oocyte prior to DNA purification. The exogenous DNA was recovered at a uniform efficiency of ~ 60%. This indicates that there is variable recovery of genomic DNA (and perhaps mtDNA) from individual oocytes that cannot be attributed to the conditions of purification or amplification. The exogenous control data enabled us to correct for inter-oocyte differences in DNA recovery, enabling an accurate quantification of mtDNA content to be carried out.

INTERACTION BETWEEN RETINOIC ACID AND PLATELET DERIVED GROWTH FACTOR RECEPTOR PATHWAYS DURING TESTICULAR GONOCYTE AND EMBRYONIC CARCINOMA CELL DIFFERENTIATION

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In an effort to understand how testicular gonocytes differentiate to spermatogonial stem cells (SSC), we recently reported that retinoic acid (RA) increases the expression of a variant PDGFRβ (V1-PDGFRβ), concomitantly with increases in the spermatogonial markers c-kit and Stra8 and a decrease in the SSC marker GFRα1. V1-PDGFRβ and PDGFRa mRNAs expression were also increased in RA-treated embryonic teratocarcinoma F9 cells concomitant with increases in the differentiation markers collagen IV and laminin B1. Here, we report that RA induces the time-dependent formation of a 45 kDa PDGFRα variant protein as well as ERK1/2 phosphorylation in F9 cells. Inhibition of PDGFR tyrosine kinase activity reduced the effects of RA on the mRNA expression of the differentiation markers. The inhibitor AG-370 also blocked RA-induced increase in PDGFRα mRNA,as well as the formation of 45 kDa PDGFRα protein and ERK phosphorylation in F9 cells. MEK/ERK inhibition with UO126 significantly decreased the effects of RA on differentiation markers and PDGFRα mRNA expression by more than 50%, but did not block the RA-induced formation of 45 kDa PDGFRα protein. Phospholipase C gamma and PI3-kinase inhibitors had no effect on any of these responses. In F9 cells, V1-PDGFRβ overexpression inhibited the RA-induced increase in PDGFRα mRNA and ERK activation, leading to an accumulation of unphosphorylated ERK in the cytosol. Gonocyte differentiation shared some similarities with the F9 model since the inhibition of PDGFR by AG-370 blocked RA-induced c-kit and Stra8 expression. However, MEK/ERK inhibition had no effect on RA-induced c-kit and Stra8 expression. RA-induced decrease in GFRα1 was not affected by PDGFR nor MEK/ERK inhibition. Altogether, these results suggest that RA and PDGFR pathways interact during gonocyte and F9 cell differentiation, involving the formation of variant PDGFRs in both cell types, but using different PDGFR downstream pathways. Since we found in another study that ERK1/2 participates in PDGF and estradiol-induced proliferation in gonocytes, it is likely that a different PDGF-induced downstream pathway is at play during gonocyte differentiation.

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DEVELOPMENT OF A SHORT-TERM FLUORESCENCE-BASED ASSAY TO ASSESS THE TOXICITY OF ANTICANCER DRUGS ON RAT SPERMATOGONIAL STEM CELLS *IN VITRO*[1]

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Spermatogonial stem cell transplantation is the gold standard assay for unequivocally measuring the stem cell activity of a given germ cell population, based on the restoration of spermatogenesis within a recipient testis; however, SSC transplantation is a time-consuming and labor-intensive procedure not amenable for screening strategies. Recently, SSC culture systems were developed for various species and have proven to be excellent *in vitro* techniques to expand and maintain SSCs as germ cell clusters over long periods of time. In the present study, we demonstrate that once formed, these germ cell clusters can be expanded then pooled in sufficient quantity for subsequent toxicological evaluation of anticancer agents. Testes from postnatal day 7/8 transgenic rat pups which express enhanced green fluorescent protein (EGFP) only in the germ cell lineage were used to rapidly purify a pure population of undifferentiated type A spermatogonia by FACS. Sorted $EGFP^{(+)}$ spermatogonia were cultured in a chemically defined serum-free medium supplemented with GDNF, GFRa1 and bFGF that formed tightly packed and round three-dimensional aggregates termed germ cell clusters after 6 days. Cultured rat $EGFP^{(+)}$ clusters were then used to assess the cytotoxic effects of cisplatin and etoposide *in vitro*. Dose-response curves were obtained for the anticancer drugs tested. Monitoring of the cluster numbers and size parameters through EGFP expression analysis were fulfilled by using fluorescence technology instrumentation; thus, very appealing for screening applications. Here, we provide evidence that the SSC cluster culture system is suitable for testing the cytotoxic effects of anticancer agents on survival and proliferation of SSC clusters. Furthermore, this culture system may offer a valuable *in vitro* model to screen libraries of small molecules for the identification of compounds that could potentially regulate self-renewal and differentiation of SSCs to further unraveling the regulation of stem cell fate.

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THE ROLE OF NODAL IN THE UTERUS DURING PREGNANCY

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Pre-term birth (PTB) is the leading cause of perinatal mortality, accounting for over 75% of perinatal death. Despite recent progress, PTB has continued to rise over the years and remains an important clinical dilemma in both developing and developed countries. Failure to decrease the rates of PTB is attributed in part to our lack of understanding of the causes and mechanisms that underlie preterm delivery. Recently, our laboratory has been characterizing the expression of the TGF-β superfamily member, Nodal, in the uterus and investigating the potential role this factor may play in facilitating the birth of healthy offspring. Utilizing the LacZ reporter system, we have shown that Nodal is expressed in the glandular epithelium throughout the uterus during the peri-implantation period. However, on day 4.5 of mouse development, which coincides with the time of blastocyst implantation, whole-mount staining generated a banding pattern along the proximal-distal axis of the uterine horns. Additional analysis has demonstrated this pattern is exclusive to day 4.5 of naturally mated mice and requires the presence of an activated blastocyst, thereby suggesting Nodal may play an active role in the implantation process. In order to bypass embryonic lethality and evaluate the potential role of Nodal in the uterus, we generated a conditional knockout of Nodal in the female reproductive tract of adult mice utilizing the loxP-Cre recombinase system. Interestingly, the Nodal deficient mice experience IUGR and fetal abortion late into development (d17.5). Therefore, we hypothesize that Nodal signalling plays a crucial role during implantation, decidualization, and trophoblast invasion and its absence leads to embryonic loss, pre-eclampsia and pre-term birth.

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ACROSIN AND MATRIX METALLOPROTEASE, MMP-2, ARE THE MAJOR PROTEINASES ASSOCIATED WITH THE INNER ACROSOMAL MEMBRANE, THE LEADING EDGE OF SPERM DURING ZONA-PENETRATION.

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During mammalian fertilization the spermatozoon penetrates the egg's zona pellucida only after a compulsory binding step occurs between the exposed inner acrosomal membrane (IAM) and the zona surface. Since the content of the acrosome has already dissipated, enzymes on the IAM surface are most likely engaged in lyses required for sperm-zona penetration. Based on this hypothesis the objective of this study was to identify IAM bound proteinases that may be involved in sperm-zona penetration. Our approach was to obtain non-ionic detergent extracts from the exposed IAM of sperm head fractions. Prospective proteinases from these extracts were identified by their hydrolyzing ability on casein and gelatin zymograms. Five digestive bands were identified of which three immunoreacted with anti-acrosin antibody while the other two were identified as matrix metalloproteases based on the inability to digest casein and blockage of their enzymatic activity with general inhibitors of metalloproteases. Immunoblotting revealed that the major metalloprotease band, of 68 kDa, reacted specifically with monoclonal antibody raised against matrix metalloprotease-2 protein (MMP-2). MMP-2 was identified in bull, swine, and mouse sperm, resulting in the first definitive identification of this proteinase in the mammalian sperm head. Our results suggest that in addition to the serine protease, acrosin, the metalloprotease, MMP-2, may play a vital role in sperm-zona pellucida penetration. Immunocytochemical studies are required to confirm the localization of MMP-2 within the sperm acrosome, while inhibition studies with specific MMP-2 inhibitors are required to provide proof of MMP-2's involvement in zona-pentration and fertilization (Supported by NSERC).

AN *EX VIVO* MODEL OF PLACENTAL DEVELOPMENT

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The placenta is an organ that facilitates nutrient and gas exchange between the mother and the fetus. It does this through highly branched embryonic blood vessels called villi. In the mouse, villi start to form when the allantois attaches to the chorion. This attachment is mediated through a thin layer of mesothelium on the chorion, which becomes indistinguishable from the allantoic cells after attachment. Subsequently, the allantois starts to form embryonic blood vessels, which enters the chorion. Abnormalitites in placental development underlie human pathologies such as pre-eclampsia, miscarriages, and intra-uterine growth retardation. The genetic and structural features of the human placenta are conserved in mice, and the murine placenta is well characterized *in vivo*. However, there are no systems to study and manipulate the placenta *ex vivo*. We hypothesize that if we culture pre-attachment chorions, and place pre-attachment allantoises on top, they will mimic early events in placental development.

Chorions and allantoises of wild type mouse embryos were dissected at somite stage 3 to 6, and were combined and cultured for 12, 24, and 36 hours *ex vivo*. We characterized changes in morphology by sectioning tissues and staining with antibodies.

We observed that the chorion was morphologically intact after culture, and the allantois differentiated and formed embryonic blood vessels. Similar to *in vivo*, the allantois attached to the chorion, and the mesothelium was not distinguishable from the allantois.

In our study, we were able to obtain an *ex vivo* model for chorion-allantois attachment. We are currently investigating whether genes required for placental development are properly expressed, and whether the villi start to form. This model will further our knowledge of early placental development as it can be easily manipulated through changing culture conditions, combining tissues from different genetic backgrounds, and transfecting the tissues prior to culturing them.

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FOLLICLE-STIMULATING HORMONE AFFECTS FOLLICULAR GROWTH BUT NOT OOCYTE GROWTH IN THE MOUSE OVARY

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Characterizing the role of follicle-stimulating hormone (FSH) on oocyte growth has many clinical applications, including improving in-vitro maturation (IVM) culture conditions. While it has been established that FSH is required for late follicular stage development, a consensus on its direct role on oocyte growth has not been reached. In this study, the effect of FSH on number of follicles in the ovary, percentage of follicles at each stage of growth, and on oocyte diameter were used as parameters of follicular and oocyte growth. FSHβ knock out mice, which do not produce any FSH, were obtained from Jackson Laboratories. Heterozygous mice were bred and the ovaries of littermates of each genotype were embedded and sectioned. Follicle counts and oocyte diameter measurements were done on H&E stained slides using a Zeiss Laser Scanning Microscope 510. The ovaries of 12-, 18-, 24- day old mice and adult mice will be compared to establish the effect of FSH during development. Preliminary results of 12- and 18-day old mice and adult mice are presented. The ovaries of FSH mutant adult mice did not contain antral follicles and most follicles were in the primary stage. In contrast, wild type adult ovaries had antral follicles and were most abundant in secondary stage follicles. Despite these differences, the average oocyte diameter of each follicle stage was similar in wild type and mutant adult ovaries. Thus far, oocytes in FSH knock out ovaries are able to reach their full size, despite being less frequent. This difference in frequency is explained by follicles' requirement of FSH in order to reach the antral stage. The similar oocyte size in mutant and wild type ovaries suggests that FSH does not affect oocyte growth. Further experiments on the ovaries at various stages of development are needed.

CHARACTERIZATION OF THE EXPRESSION AND REGULATION OF PANNEXIN-1 AND PANNEXIN-3 IN THE MALE REPRODUCTIVE TRACT OF ADULT RAT.

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A novel family of gap junction (GJ) proteins, pannexin (panx), was recently identified in vertebrates. Although homologous to invertebrate GJ proteins, the innexins, their function in mammals remains obscure. To evaluate the role of panxs in the male reproductive tract, we investigated the expression and distribution of panx-1 and -3 in the testis, efferent ducts, and epididymis of adult rat. In the testis, panx1 was localized to the basal compartment of seminiferous epithelium. Panx3 labelling was punctate in areas of Leydig cells. In efferent ducts, both panx1 and panx3 were expressed in the epithelium, notably at the apical region of ciliated cells. In the epididymis, both panxs were expressed in principal cells. Prominent labelling for panx1 was also observed between principal and basal cells. Panx3 was also found at the plasma membrane of halo cells. Western blots confirmed the expression of both panxs in the male reproductive tract, where various isoforms were detected, corroborating previous reports of post-translational glycosylation. However, RT-PCR results suggest that alternative splicing may account for some of these isoforms. Panx3, which appears to be regulated by androgens, was shown to be more abundant in proximal epididymal regions, whereas panx1 was expressed at a relatively constant level throughout the epididymis. In conclusion, panxs are expressed throughout the male reproductive tract in a cell-specific manner. Moreover, the differential expression of panx3 in the epididymis and its responsiveness to androgens suggest that it may be tightly regulated within this tissue and may thus play a role in epididymal sperm maturation.
Supported by NSERC and CIHR.

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FOLATE DEFECIENCY IN UTERO AND POSTNATALLY IMPAIRS SPERMATOGENESIS, FERTILITY AND EPIGENETIC PROGRAMMING IN A MOUSE MODEL

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It is estimated that large numbers (10-70%) of populations in developed countries are not consuming the recommended dietary amounts of folate. Previous studies have shown that folate is a determinant of male reproductive health but the underlying molecular mechanisms linking spermatogenesis, fertility and folate are unknown. Spermatogenesis is characterized by massive alterations to the epigenome and our previous work described a tightly regulated pattern of histone H3 methylation in germ cell development that coincides with highly regulated transcription and cell differentiation. Epigenetics refers to heritable changes in gene expression that are regulated by DNA methylation and the covalent modification of histones. Given that dietary folate can alter the supply of methyl donors, we hypothesized that male germ cells may be sensitive to dietary folate and that this may manifest as altered epigenetic programming and have downstream consequences spermatogenesis and fertility. The objective of this study was to determine the impact of exposure to a low folate diet, during embryonic development and into adulthood on histone methylation, spermatogenesis and fertility. C57/BL6 females were fed either a folate-sufficient (FS, 2 mg of folate/kg of diet), or a folate-defecient diet (FD, 0.3 mg of folate/kg of diet) two weeks prior to breeding, through pregnancy and lactation. Weaned male pups received the same diet as their mother until sacrifice. Testes were collected at postnatal days 6-18 and from sexually mature males at 6 weeks and 15 weeks of age. In comparison to the FS group, the FD group had decreased epididymal weight, increased germ cells apoptosis, abnormal spermatogenesis and 86% were infertile. Epigenetic programming was disturbed in the folate depleted mice with a reduction in gene activating histone H3-lysine 4 tri-methylation. This is the first study to characterize the impact of folate deficiency on spermatogenesis and the epigenetic layer in spermatogenesis. The results suggest that epigenetic programming during spermatogenesis is sensitive to dietary levels of folate.

ACCUMULATION OF THE STEM-LOOP-BINDING PROTEIN DURING MEIOTIC MATURATION REQUIRES ON-GOING PROTEASOMAL ACTIVITY

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The stem-loop-binding protein (SLBP) regulates the post-transcriptional processing and translation of non-polyadenylated histone mRNAs. SLBP is synthesized in fully grown immature oocytes, and corresponding degradation of the protein maintains a steady-state level. During meiotic maturation, translational activation of its mRNA leads to a substantial accumulation of SLBP. Translational activation is accompanied by an increase in the average length of the poly(A) tail. The 3′-utr of SLBP mRNA contains sequences that resemble cytoplasmic polyadenylation elements (CPEs), and deletion of these increases the translation of reporter mRNAs in immature oocytes and decreases their translation in maturing oocytes. Blocking proteasomal activity in maturing oocytes, even after SLBP has begun to accumulate, prevents further accumulation of the protein. This requirement for proteasomal activity persists after at least 80% of the CPE-binding (CPEB) protein in the oocyte has been degraded. These results indicate that CPE sequences can regulate the translation of mRNAs that are expressed in both immature and maturing oocytes, and reveal an unexpected role for on-going proteasomal activity in regulating protein synthesis during oocyte maturation.

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THE KRLN MOTIF OF IAM38 IS INVOLVED IN SPERM-ZONA SECONDARY BINDING DURING FERTILIZATION

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IAM38/SP38 is a prominent protein of the inner acrosomal membrane extracellular coat (IAMC) of spermatozoa retained on the inner acrosomal membrane after acrosomal exocytosis (Yu et al., Dev. Biol., 2006). We have shown that IAM38 occupies the leading edge of sperm contact with the zona pellucida of the oocyte during fertilization, and that secondary binding and porcine fertilization were inhibited *in vitro* by antibodies directed against IAM38. In this study we were interested in resolving the mechanism used by IAM38 to attach the sperm to the zona pellucida. Utilizing mouse *in vitro* fertilization as our model, we provide evidence that the synthetic peptide derived from the ZP2-binding motif of IAM38, KRLSKAKNLIE, has a competitive inhibitory effect on both sperm-zona binding and fertilization while its mutant form, in which two basic amino terminal residues were substituted for by acidic residues was ineffective. A similar blocking effect on secondary binding and mouse fertilization was achieved with antibodies directed against IAM38. These results bolster our hypothesis that IAM38 is an important player in secondary binding during mammalian fertilization and identify the KRLXX(XXX)LIE sequence of IAM38 as at least partially responsible for the secondary binding that exists between sperm and zona-pellucida during *in vitro* fertilization.

COMPARATIVE PROTEOMICS ANALYSIS BETWEEN LEFT AND RIGHT SIDES OF CHICK EMBRYOS

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In vertebrate, it is critical for normal physiological function that many internal organs are asymmetrically positioned with respect to the midline. In humans, the occurrence of laterality defects is 1:10000 in live births and there is an increased incidence of congenital anomalies among first degree relatives of individuals with situs defects. The chick embryo has been extremely useful to identify many of the molecules that direct patterning of the left-right (L-R) axis during embryogenesis. However, asymmetric gene expression observed in the early embryo failed to provide a comprehensive understanding of morphogenetic movements that result in asymmetric organogenesis. We investigated the proteomic profiles of left and right sides of HH stage 9-10 chick embryos to identify additional proteins that are asymmetrically expressed and required for normal patterning of the L-R axis. Protein extracts were prepared from the left and right lateral halves of HH stage 10 chick embryos, subjected to SDS-PAGE on a 7-15% gradient gel which was sliced into 15 bands. In-gel tryptic digests were performed and the peptides were subjected to tandem ion-trap mass spectrometer (MS) (MS/MS) analyses. Two rounds of proteomic screening have been performed. Preliminary analysis has identified 32 candidates showing a score ≥ Mascot ID score that have a fold change ≥2 that were differentially expressed between the left and right halves of the embryo. In addition, 35 of candidates were expressed only on the left side and 7 were expressed only on the right side. Asymmetric expression patterns will be confirmed using immunohistochemistry and western blot assay. In addition, we will determine if the asymmetric expression of these candidates is regulated at the level of transcription or translation. Functional assays will be performed for those candidates that are asymmetrically expressed in the lateral plate mesoderm and/or in primordia of the organs that are asymmetrically positioned.

BREAKTHROUGHS IN REPRODUCTION & DEVELOPMENT

Research Day of the Centre for the Study of Reproduction (CSR) at McGill &
the Human Reproduction and Development axis of the MUHC
May 7, 2009 - 8:45 a.m. - 5:00 p.m.

List of attendees / Liste des participants

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

Breakthroughs in reproduction and development

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

Palmer Howard Amphitheatre, 6th Floor, McIntyre Medical Building, McGill University

Thursday, May 7, 2009

EVENT EVALUATION SURVEY

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

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

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

Comments/Suggestions:

McGill

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

Nouvelles avancées en reproduction et développement

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

Amphithéâtre Palmer Howard, 6e étage, Édifice McIntyre, Université McGill

Le jeudi 7 mai 2009

FORMULAIRE D'ÉVALUATION

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

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

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

Commentaires/Suggestions:

McGill