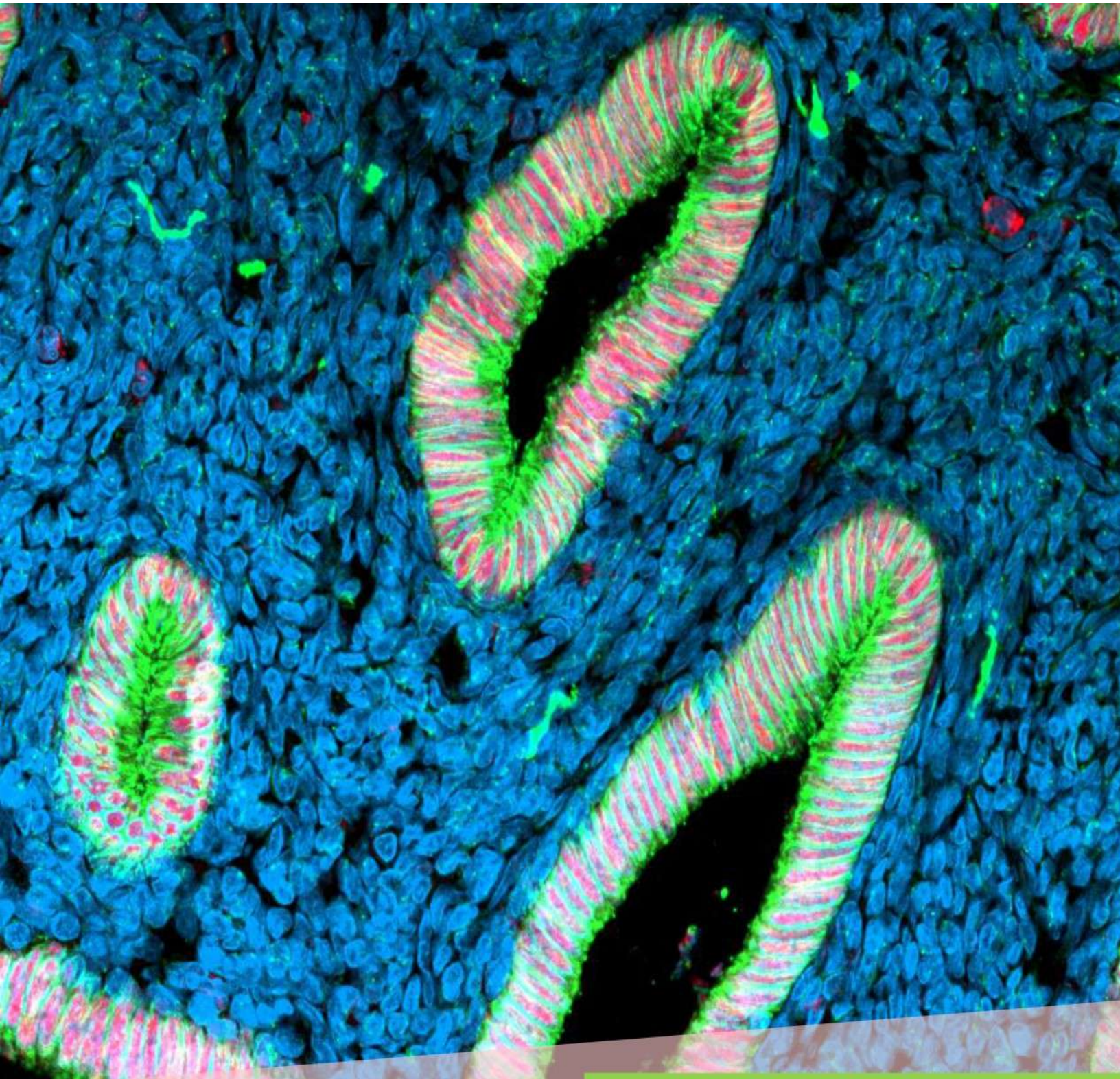


RQR

Réseau Québécois en reproduction

13e Symposium annuel du Réseau Québécois en reproduction
9-10-11 novembre 2020

13th Annual Symposium of the Réseau Québécois en reproduction
November 9-10-11, 2020

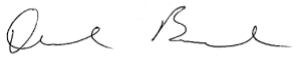
À tous les membres du RQR, collaborateurs, étudiants et invités,

Au nom du Comité exécutif du RQR, j'ai le plaisir de vous accueillir au 13ème Symposium annuel du Réseau Québécois en reproduction (RQR). Bien que la pandémie nous ait empêché de tenir le traditionnel rassemblement en personne, que nous avions initialement prévu au Centre de Recherche du CHU Ste-Justine, nous nous sommes ajustés en organisant ce que nous prévoyons être une alternative virtuelle très satisfaisante.

Tenu sur une période de trois jours avec un calendrier simple et convivial, notre Symposium virtuel présentera néanmoins tous les principaux événements auxquels vous vous attendez. Les trois conférenciers invités par nos Axes de recherche seront les Drs Thomas Spencer (University of Missouri) qui présentera « *Insights into the biological roles of uterine glands in pregnancy establishment* », Patricia Ann Hunt (Washington State University), qui discutera de « *Maternal exposure and the developing fetus* », et Pascal Gagneux (University of California) qui partagera ses travaux sur « *Gametes, glycans, and genital tracts* ». Le Dr Patrick Blondin (Semex-Boviteq), présentera le séminaire en production animale, axé cette année sur les défis et les opportunités auxquels fait face l'industrie des bovins laitiers. Nous sommes heureux d'accueillir Mme Bibiana Pulido, directrice générale du Réseau interuniversitaire québécois sur l'Équité, la Diversité et l'Inclusion, qui présentera un séminaire sur un thème d'actualité : « *Pourquoi parle-t-on d'équité, de diversité et d'inclusion (EDI) dans le milieu universitaire ? Une mode passagère ou un réel changement* ». Enfin, les Drs Greg FitzHarris (Université de Montréal) et Maria Gracia Gervasi (University of Massachusetts) dirigeront également un atelier qui présentera certaines des techniques de microscopie les plus avancées actuellement utilisées dans le domaine de la biologie de la reproduction.

En plus des conférenciers invités, nous attendons avec impatience les présentations orales et par affiches de nos étudiants et stagiaires postdoctoraux. Nouveauté cette année, une session de présentation orale réservée à quatre doctorants du GDR 3606 Repro, notre société sœur française avec laquelle nous continuons à tisser des liens d'amitié et de collaboration. Joignez-vous à nous pour leurs présentations, montrant les plus récents travaux en biologie de la reproduction du vieux continent. Nous espérons sincèrement que vous apprécierez les trois prochains jours de science dans le confort de votre foyer et nous avons hâte de vous voir l'année prochaine lorsque le Symposium reviendra au Centre des Congrès de Québec.

Au plaisir d'interagir avec vous tous, Daniel Bernard, Directeur du RQR



To all RQR members, collaborators, trainees, and guests,

On behalf of the RQR Executive Committee, it is my pleasure to welcome you to the 13th Annual Symposium of the Réseau Québécois en reproduction (RQR). Although the pandemic has precluded us from holding the traditional, in-person gathering that we had originally planned for Centre de Recherche du CHU Ste-Justine, we have adjusted by organizing what we expect to be a very satisfying virtual alternative.

Held over a three-day period and with a breezy, user-friendly schedule, our virtual Symposium will nonetheless feature all of the main events you've come to expect. Our three Axis speakers will be Dr. Thomas Spencer (University of Missouri) who will present "*Insights into the biological roles of uterine glands in pregnancy establishment*", Dr. Patricia Ann Hunt (Washington State University), who will discuss "*Maternal exposure and the developing fetus*", and Dr. Pascal Gagneux (University of California) who will share his work on "*Gametes, glycans, and genital tracts*". Knowledge Transfer Committee invitee Dr. Patrick Blondin (Semex-Boviteq) will present the animal production seminar, this year focused on the challenges and opportunities faced by the dairy cattle industry. We are pleased to welcome Ms Bibiana Pulido, the Executive Director of the Québec Interuniversity Equity, Diversity and Inclusion Network, who will present a seminar on the timely topic of "*Pourquoi parle-t-on d'équité, de diversité et d'inclusion (ÉDI) dans le milieu universitaire? Une mode passagère ou un réel changement*". Finally, Drs Greg FitzHarris (Université de Montréal) and Maria Gracia Gervasi (University of Massachusetts) will also direct a workshop that will present some of the most cutting-edge microscopy techniques currently being used in the field of reproductive biology.

In addition to the invited speakers, we look forward to oral and poster presentations from our trainees. New this year will be an oral presentation session reserved for four PhD trainees from the GDR 3606 Repro, our French sister society with which we continue to build ties of friendship and collaboration. Please join us as they present some of the newest reproductive biology work from the old continent. We sincerely hope you will enjoy the next three days of science from the comfort of your own home, and look forward to seeing you next year when the Symposium will return to the Centre des Congrès in Quebec City.

All the best, Daniel Bernard, RQR Director

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Agenda of the 13th Symposium of the Réseau Québécois en reproduction

Lundi 9 novembre – *Monday November 9th*

9h00 – 9h05	Mot de bienvenue *Welcome*
9h05 – 10h20	Présentations : Session I *Presentations: Session I*
10h20 – 11h00	Séminaire en production animale/*Animal production seminar* *** Patrick Blondin (Semex-Boviteq) *Mise à jour sur l'innovation et les défis de l'industrie bovine*
11h00 –15h00	Pause *Break*
15h00 – 15h30	Atelier/*Workshop*: Greg FitzHarris (Université de Montréal) *Horses for courses - choosing the right microscope for reproductive biology experiments*
15h30 – 16h00	Atelier/*Workshop*: Maria Gracia Gervasi (University of Massachusetts) *Unraveling the sperm actin cytoskeleton by superresolution microscopy*
16h00 – 16h30	Table ronde avec les 2 conférenciers */ Panel discussion with the 2 speakers*
16h30 – 17h15	Conférencier invité/*Invited Speaker* *** Pascal Gagneux (University of California) *Gametes, Glycans, and Genital Tracts*

Mardi 10 novembre - *Tuesday November 10th*

9h00 – 9h05	Bienvenue/introduction *Welcome/introduction*
9h05 – 10h00	Séminaire EDI/*EDI seminar* *** Bibiana Pulido (Université de Montréal) *Pourquoi parle-t-on d'équité, de diversité et d'inclusion (ÉDI) dans le milieu universitaire? Une mode passagère ou un réel changement?*
10h00– 11h00	Présentations des étudiants du GDR 3606 repro */ GDR 3606 repro student presentations*
11h00 – 15h00	Pause *Break*
15h00 – 16h15	Présentations: Session II *Presentations: Session II*
16h15 – 17h00	Conférencière invitée/*Invited Speaker* *** Patricia Ann Hunt (Washington State University) *Maternal exposure and the developing fetus*

Mercredi 11 novembre - *Wednesday November 11th*

9h00 – 9h05	Bienvenue/introduction *Welcome/introduction*
9h05 – 10h20	Présentations: Session III *Presentations: Session III*
10h20 – 11h05	Conférencier invité/*Invited Speaker* *** Thomas Spencer (University of Missouri) *Insights into the Biological Roles of Uterine Glands in Pregnancy Establishment*
11h05 – 11h15	Remise des prix/*Awards* Fermeture de session/*Closure session*

Présentations – Presentations : Session I
9 novembre – November 9th
9h05 – 10h20

I. **Characterization of a novel inhibin B co-receptor**
Emilie Brûlé, PhD Student, McGill University (Page 7)
9h05 – 9h20

II. **Effects of an environmentally relevant mixture of organophosphate esters on testis function and sperm capacitation in rats**
Aimee Katen, Postdoctoral Fellow, McGill University (Page 8)
9h20 – 9h35

III. **A new role for Gata4 in hypothalamus development and PCOS pathogenesis**
Sherin Nawaito, Postdoctoral Fellow, UQAM (Page 9)
9h35 – 9h50

IV. **Potential involvement of the Hippo pathway in mediating the LH response in ovarian granulosa cells**
Philippe Godin, PhD Student, Université de Montréal (Page 10)
9h50 – 10h05

V. **Action antitumorale de la mélatonine dans le choriocarcinome placentaire: rôle du stress du réticulum endoplasmique**
Josianne Bienvenue-Pariseault, PhD Student, INRS (Page 11)
10h05 – 10h20

Characterization of a novel inhibin B co-receptor

Brûlé E.[1], Wang Y.[2], Ongaro L.[2], Lin Y-F.[2], Li Y[2]. Zhou X.[2], Bernard D.J.[1,2]

Departments of [1]Anatomy and Cell Biology, [2]Pharmacology and Therapeutics, McGill University, Montreal, Canada

Follicle-stimulating hormone (FSH) is essential for mammalian reproduction, particularly in females. FSH production in pituitary gonadotropes is dynamically regulated by multiple factors, including gonadal inhibins. Inhibins are members of the transforming growth factor β (TGFβ) family that act as competitive antagonists to intra-pituitary activins. Inhibins bind to activin type II receptors in concert with a co-receptor, forming a ternary complex. Until recently, betaglycan (*Tgfbr3*) was considered the obligate co-receptor for both inhibin A and B. However, pituitary cells from gonadotrope-specific betaglycan knockout mice remain sensitive to inhibin B, suggesting that there may be an additional co-receptor for inhibin B.

We recently discovered a novel protein, inhibin B binding protein (IBBP), that we hypothesize is the inhibin B specific co-receptor. In heterologous cells, inhibin B, but not inhibin A, sensitivity is enhanced by transfection of IBBP. In homologous cells, IBBP knockdown attenuates inhibin B responses. *Ibbp* global knockout females are supra-fertile, ovulating more eggs per cycles and producing approximately three more pups per litter than controls. However, their pituitaries remain sensitive to both inhibin A and B. We next generated animals with a global deletion of *Ibbp* and gonadotrope-specific ablation of betaglycan (hereafter, dcKO). dcKO females have markedly elevated eggs ovulated per cycle naturally and have ovaries that are four times heavier than those of littermate controls; yet, these females to not deliver live offspring. Importantly, dcKO pituitaries are insensitive to both inhibin A and B. These results demonstrate that IBBP is an inhibin B co-receptor.

Effects of an environmentally relevant mixture of organophosphate esters on testis function and sperm capacitation in rats

Aimee L. Katen[1], Abishankari Rajkumar[1], Trang Luu[1], Xiaotong Wang[1], Zixuan Li[1], Dongwei Yu[1], Elanie Yip[1], Michael G. Wade[2], Barbara F. Hales[1] and Bernard Robaire[1,3,4]

[1]Department of Pharmacology & Therapeutics, McGill University, Montréal, Québec, Canada, [2]Environmental Health Science & Research Bureau, Health Canada, Ottawa, Ontario, Canada, [3]Department of Obstetrics and Gynecology, McGill University, Montréal, Québec, Canada, [4]The Research Institute of the McGill University Health Centre, Montréal, Québec, Canada

Despite ubiquitous human exposure, there are limited data on the effects of organophosphate esters (OPEs) on reproductive function. We tested the effects of the combination of OPEs detected in Canadian house dust on male reproduction.

Male and female rats (n=15/treatment) were administered vehicle or OPE mixture, low(L), middle(M) or high(H) dose, via the diet for 70 and 30 days, respectively, approximating 30, 1,000 or 30,000 times the levels humans are exposed to via dust. Following mating, female rats continued treatment throughout gestation and lactation, when pups were transferred to an OPE-free diet and euthanized on PND 90.

OPE exposure of F0 males had no effect on testosterone levels, sperm production, motility, levels of DNA damage or lipid peroxidation. However, the L group had an increased percentage of cauda epididymal sperm that were acrosome reacting/reacted. Following 3h incubation in capacitating media, sperm from this group had increased levels of phosphotyrosine fluorescent staining (225% of control), an indication of sperm capacitation status. In F1 rats, daily sperm production was reduced by 31 and 36% in the M and H groups. An increase in 4-hydroxynonenal immunofluorescence was detected in the M and H groups (190% and 202% of control). Increased DNA damage and phosphotyrosine staining were observed in the L group.

Thus, exposure of adult males to an environmentally-relevant mixture of OPEs disrupted sperm capacitation and the acrosome reaction. Impaired sperm production, increased oxidative stress, DNA damage and changes to capacitation status were observed in the F1 generation. Funded by CIHR and FRQS.

A new role for Gata4 in hypothalamus development and PCOS pathogenesis

Nawaito SA[1,2], Esmael M[1,2], Bernas G[1,2], Bergeron K-F[2,3], Souchkova O[1,2], Gayda F[1,2], Bergeron F[4], Viger RS[4], Mounier C[2,3] and Nicolas Pilon[1,2]

[1]Molecular Genetics of Development Laboratory, Department of Biological Sciences, Faculty of Sciences, University of Quebec at Montreal (UQAM) [2] Centre d'Excellence en Recherche sur les Maladies Orphelines – Fondation Courtois, UQAM, [3] Lipid Metabolism Laboratory, Department of Biological Sciences, Faculty of Sciences, UQAM.[4] Reproduction, Perinatal and Child Health, Centre de Recherche du Centre Hospitalier Universitaire de Québec (CRCHUQ), Centre de recherche en Reproduction, Développement et Santé Intergénérationnelle (CRDSI) and Department of Obstetrics and Gynecology, Faculty of Medicine, Laval University.

Polycystic Ovary Syndrome (PCOS), a complex hormonal disorder, is the leading cause of female infertility. Its etiology remains unknown and current management remains palliative. However, neural crest-derived gonadotropin-releasing hormone (GnRH) neurons located in the hypothalamus are suspected to play a role in PCOS pathogenesis.

We generated a transgenic mouse model, named *Greywick* (*Gw*) that exhibits subfertility and obesity specifically in females by insertion of neural crest-specific *Gata4* promoter-driven *RFP* reporter in the pseudogene *Gm10800*. Our data show that adult *Gw* females have increased serum levels of LH and estrogen and increased expression levels of *Gnrh*, with concomitant decreases in expression levels of the corresponding receptors in the hypothalamus. Moreover, we discovered that *Gw* females are obese because they eat more. Accordingly, serum levels of leptin, a key regulator of food intake, were also found to be increased in *Gw* females. Treatment with metformin and TUDCA increase the number and viability of oocytes. The normal phenotype of mice with CRISPR-mediated knockout of *Gm10800* suggests that the PCOS phenotype of *Gw* mice is due to knockdown of endogenous *Gata4* through promoter competition. Accordingly, *Gata4* is downregulated in the hypothalamus of *Gw* females but not in ovaries. *Gw* mice might thus help to explain the recent association of a *GATA4* single nucleotide polymorphism with human PCOS. Our goal is to understand the mechanism by which Gata4 regulates the hypothalamic-pituitary-gonadal axis in the context of PCOS.

Potential involvement of the Hippo pathway in mediating the LH response in ovarian granulosa cells

Philippe Godin[1], Mayra Tsoi[1], Martin Morin[2], Nicolas Gévry[2] and Derek Boerboom[1]

[1]Centre de Recherche en Reproduction et Fertilité (CRRF), Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, QC, Canada, [2]Département de Biologie, Faculté des Sciences, Université de Sherbrooke, Sherbrooke, QC, Canada.

The LH surge is a pivotal event that triggers multiple ovarian processes including oocyte maturation, cumulus expansion, follicular wall rupture and luteinization of mural granulosa and theca cells. Binding of LH to its receptor stimulates several downstream signalling pathways, but our understanding of the mechanisms of LH action remains incomplete. Recently, gene targeting of the Hippo pathway was shown to downregulate *Areg* and *Egfr*, two components of the LH-induced EGFR pathway, suggesting Hippo as a potential regulator of the LH cascade. This study aims to better characterize the involvement of Hippo in the LH response in murine granulosa cells. Cells treated with LH *in vitro* or collected from immature mice treated with eCG and hCG showed a transient increase in phospho-LATS1(Thr1079), phospho-YAP(Ser127 and Ser397), and phospho-TAZ(Ser89) levels. *In vitro* pharmacologic inhibition revealed that Hippo activation by LH is PKA-dependent. Knockdown of the Hippo transcriptional co-regulators *Yap* and/or *Taz* blunted the induction of several LH targets such as *Areg*, *Btc*, *Pgr*, *Ptgs2*, *Star* and *Tnfaip6* and prevented the activation of the ERK1/2 pathway. Microarray analyses revealed substantial overlap between genes that are upregulated by LH and those that are downregulated following loss of *Yap*/*Taz*, suggesting a major role for Hippo in mediating LH action. Many *Yap/Taz*-regulated genes were related to biological processes regulating ovulation. Using ChIP-qPCR analyses, we are currently investigating the potential direct transcriptional regulation of *Areg* by YAP/TAZ. Overall, these results establish Hippo as a pathway that is activated by LH and that is required to mediate its actions.

Action antitumorale de la mélatonine dans le choriocarcinome placentaire: rôle du stress du réticulum endoplasmique

Josianne Bienvenue-Pariseault[1,2], Darius E. Stamatakos[3], Cathy Vaillancourt[1,2]

[1]INRS – Centre Armand-Frappier Santé Biotechnologie, [2]Réseau intersectoriel en santé de l'Université du Québec (RISUQ), [3]Université d'Ottawa.

Contexte : Par une augmentation du stress oxydatif (SO), la mélatonine induit la voie des protéines mal repliées (UPR) et l'apoptose dans différents cancers faisant d'elle un adjuvant prometteur. Cependant, ceci n'a pas encore été étudié dans les cellules de choriocarcinomes placentaires BeWo. ***Hypothèse*** : La mélatonine exerce une action antitumorale dans les cellules BeWo. ***Objectifs*** : Déterminer dans les BeWo, si la mélatonine a un effet 1) anti-apoptotique; 2) activateur du stress du RE et 3) pro-oxydatif. ***Méthodologie*** *:* Les cellules BeWo ont été exposées à la mélatonine (1 mM) sous normoxie (8 %-O_2). L'apoptose a été détectée par FACS (annexine-V). Les niveaux protéiques et d'ARNm des facteurs impliqués dans le stress du RE, l'apoptose et le SO ont été analysés respectivement par immunobuvardage et RT-qPCR. Le SO a été mesuré par spectrofluorométrie (carboxyH2DCFDA, monochlorobimane). ***Résultats*** *:* Dans les cellules BeWo, la mélatonine, comparée au contrôle (DMSO), augmente significativement 1) les taux protéiques de PARP-clivé (*74%, t-test, P=0,0089*); 2) les taux protéiques de GRP78 (*167%, t-test, P=0,0222*) et des facteurs impliqués dans la voie PERK-UPR alors qu'elle n'a pas d'effet sur les facteurs de la voie IRE1α-UPR; et 3) le SO. Des essais préliminaires ont démontré que la mort des cellules traités avec la mélatonine et le méthotrexate (100 nM) étaient plus élevés que celle des cellules traitées uniquement au méthotrexate (-32%, *Kruskal-wallis, p=0,0369*). ***Conclusion*** : Cette étude suggère que l'action antitumorale de la mélatonine dans le choriocarcinome placentaire est médiée par la voie du stress du RE UPR-PERK.

Conférencier invité - Invited Speaker

Patrick Blondin

Le Dr Patrick Blondin est à l'avant-scène de l'innovation en recherche, de la découverte et de la croissance des marchés depuis plus de 15 ans. Il tisse des partenariats critiques avec gouvernements, universités, partenaires industriels & financiers. Il a un rôle primordial dans la mise en place du seul Centre canadien en reproduction assistée pour le bovin, ainsi que des laboratoires FIV sur 3 continents. Il est également l'auteur de plus de 75 articles dans des journaux scientifiques en reproduction et génétique.

Il présentera un séminaire en production animale, le lundi 5 novembre intitulé : « ***Mise à jour sur l'innovation et les défis de l'industrie bovine »***.

Atelier du RQR - RQR workshop

Conférencier invité - Invited Speaker

Greg FitzHarris

Greg's lab is interested in cell divisions in the niche and clinically highly important setting of the mammalian oocyte and early embryo. His lab has used a variety of microscopy types and approaches to tackle this question over the past 13 years, and has been involved in a number of microscopy 'firsts' in this setting. Greg will discuss some of the different approaches commonly available in many research centers, and try to explain that choosing the most appropriate - rather than necessarily the 'flashiest' - equipment and strategy for the question to be addressed is often key to experimental success. Greg is Proffessor at University of Montreal, researcher at CRCHUM, Director of UdeM's Centre for Research in Reproduction and Fertility, and Co-Editor in Chief of Reproduction.

On Monday, during the Symposium workshop, Dr. Greg FitzHarris will give a talk entitled: ***"Horses for courses - choosing the right microscope for reproductive biology experiments"***.

Atelier du RQR - RQR workshop

Conférencière invitée - Invited Speaker

Maria Gracia Gervasi

My name is Maria Gracia Gervasi and I am a Research Assistant Professor in the Department of Veterinary and Animal Sciences at the University of Massachusetts, Amherst, USA. My scientific career has been dedicated to the study of molecular pathways that regulate the sperm acquisition of fertilization competence. I obtained my Biology degree in 2007 at the School of Exact and Natural Sciences, University of Buenos Aires (FCEN-UBA, Buenos Aires, Argentina). During my last years of undergraduate studies, my interest for animal physiology and particularly biology of reproduction arose and made me pursue my Ph.D. studies in the field. In 2013 I obtained my Ph.D. in Biology also at the FCEN-UBA in Argentina. A year before graduating I was selected to attend the distinguished international course “Frontiers in Reproduction” held at the Marine Biological Laboratory (MBL) in Woods Hole (Massachusetts, USA). This was a transformative experience for my career that motivated me to move to the United States to continue my studies as a postdoctoral fellow in the laboratory of Dr. Pablo Visconti at the University of Massachusetts, Amherst. My first two years of postdoctoral studies were supported by the Lalor Foundation. During my postdoc, I developed an interest in the application of advanced microscopy techniques to answer sperm physiology related questions. As a first approach to optical microscopy, in 2014 I attended the Optical Microscopy for Biomedical Sciences course held at the MBL (Woods Hole, Massachusetts). After that I started the beautiful journey of studying the sperm cytoskeleton by super-resolution microscopy. This resulted in exciting discoveries that contributed to the field of male reproduction and fertility.

On Monday, during the Symposium workshop, Dr. Maria Gracia Gervasi will give a talk entitled: **“*Unraveling the sperm actin cytoskeleton by superresolution microscopy*”**.

Conférencier invité - Invited Speaker

Pascal Gagneux

Pascal Gagneux is Professor of Pathology and Anthropology at UC San Diego. He is interested in the evolutionary mechanisms responsible for generating and maintaining primate molecular diversity. His team is exploring the roles of molecular diversity in protecting populations from pathogens as well as potential consequences for reproductive compatibility. The Gagneux laboratory studies cell-surface molecules of sperm cells in closely related primates species. His focus is on glycans, the oligosaccharides attached to glycolipids and glycoproteins of the cell surface. The numerous parallels between the surface molecules of successful pathogens and those found on the surface of mammalian sperm, invite the analogy between internal fertilization and "extremely successful infection." Dr. Gagneux's interest is in how differences in sperm surface molecules reflect sexual selection (via sperm competition and cryptic female choice) and whether such differences might contribute to reproductive incompatibility and speciation due to female immune rejection of sperm decorated with incompatible glycoconjugates. The operating assumption is that glycan evolution is shaped by constraints from endogenous biochemistry and exogenous, pathogen-mediated natural selection, but could also have consequences for sexual selection. Dr. Gagneux has studied the behavioral ecology of wild chimpanzees in the Taï Forest, Ivory Coast, population genetics of West African chimpanzees, and differences in sialic acid biology between humans and great apes with special consideration of their differing pathogen regimes. His great concern is that the current surge in interest for comparative genomics is not being translated into direct support for the conservation of primates in their endangered natural habitats.

On Monday, Dr. Pascal Gagneux will give a talk entitled: ***"Gametes, Glycans, and Genital Tracts"***.

Conférencière invitée - Invited Speaker

Bibiana Pulido

Bibiana est cofondatrice et directrice générale du Réseau interuniversitaire québécois pour l'équité, la diversité et l'inclusion (RIQEDI), un OBNL regroupant les universités québécoises ainsi que plusieurs parties prenantes du milieu universitaire qui ont le mandat ou le désir de promouvoir et d'intégrer les valeurs d'équité, de diversité et d'inclusion au sein de leurs institutions. Elle assume actuellement le poste de directrice adjointe de l'Institut équité diversité inclusion intersectionnalité (Institut EDI2). Chercheure et gestionnaire possédant plus de dix ans d'expérience dans le domaine de la gestion des ressources humaines. Ses domaines d'expertise sont en lien avec l'analyse des industries innovantes, créatives et académiques, en gestion de l'équité, la diversité et l'inclusion, en gestion des conflits, en bien-être au travail ainsi qu'en gestion du transfert des connaissances (*knowledge management*) au sein des entreprises et des "clusters". Elle est candidate au doctorat en relations industrielles (spécialisation ressources humaines) et est reconnue pour son dévouement à l'enseignement, l'encadrement d'étudiant(e)s, la recherche universitaire et l'engagement communautaire.

Bibiana Pulido présentera un séminaire en Équité, Diversité et Inclusion, le mardi 10 novembre intitulé : **« Pourquoi parle-t-on d'équité, de diversité et d'inclusion (ÉDI) dans le milieu universitaire? Une mode passagère ou un réel changement »**.

Présentations – Presentations : étudiants du GDR repro/GDR repro trainees
10 novembre – November 10th
10h00 – 11h00

I. **Genetic and molecular characterization of severe forms of teratozoospermia responsible for male infertility**
Julie Beurois, PhD Student, Université Grenoble-Alpes (Page 18)
10h00 – 10h15

II. **Activité de l'ovaire au cours de la mini-puberté : mécanismes sous-jacents et rôle dans la fonction de reproduction**
Marie Devillers, PhD Student, Université de Paris (Page 19)
10h15 – 10h30

III. **Epigenetics alterations of the murine male reproductive system following gestational exposure to chlordecone**
Louis Legoff, PhD Student, Université de Rennes 1 (Page 20)
10h30 – 10h45

IV. **Régulation de l'expression de Sf-1 au cours de la différenciation du lignage gonadotrope hypophysaire**
Vincent Pacini, PhD Student, Université de Paris (Diderot) (Page 21)
10h45 – 11h00

Genetic and molecular characterization of severe forms of teratozoospermia responsible for male infertility

Julie Beurois[1], Denis Dacheux[2,3], Guillaume Martinez[1,4], Caroline Cazin[1], Zine-Eddine Kherraf[1,4], Derrick R Robinson[2], Amir Amiri-Yekta[5], Patrick Lorès[6], Raouda Zouari[7], Jessica Escoffier[1], Corinne Loeuillet[1], Nicolas Thierry-Mieg[8], Aminata Touré[1], Christophe Arnoult[1], Mélanie Bonhivers[2], Pierre F Ray[1,4], Charles Coutton[1,4].

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Teratozoospermia is defined by the World Health Organization as the presence in the ejaculate of more than 96% of morphological abnormal spermatozoa. It represents a heterogeneous group including a wide range of phenotypes including Multiple Morphological Abnormalities of the sperm Flagella (MMAF) and globozoospermia. MMAF is responsible for male infertility due to the presence in the ejaculate of immotile spermatozoa with severe flagellar abnormalities including flagella being short, coiled, absent and of irregular caliber. Globozoospermia is characterized by the presence in the ejaculate of a large majority of round spermatozoa lacking the acrosome which are unable to adhere and penetrate the oocyte's zona pellucida, causing primary infertility. Mutations in several genes encoding axoneme and acrosome-related proteins have been associated with the MMAF and globozoospermia phenotypes. However, most of the cases analyzed remain unresolved, suggesting that many yet uncharacterized gene defects account for these phenotypes. The objective of my thesis is to investigate monogenic causes of these two phenotypes. After performing and analyzing whole-exome sequencing data from a large cohort of 167 MMAF and 28 globozoospermic patients, we identified three new candidate genes in MMAF phenotype: CFAP70, MAATS1 and ZMYNDX and one candidate gene in globozoospermia: GLOBO1. Molecular analyses and physiopathological characterization using different animal models have already been performed for CFAP70(1) and MAATS1(2) genes providing new knowledge on male infertility causes, and investigations are ongoing for the other two candidate genes ZMYNDX and GLOBO1.

Activité de l'ovaire au cours de la mini-puberté : mécanismes sous-jacents et rôle dans la fonction de reproduction

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Chez les mammifères femelles, on observe, juste après la naissance, une phase d'activation transitoire de l'axe hypothalamus-hypophyse-ovaires qui correspond à la « mini-puberté ». Cela se traduit par l'élévation des niveaux circulants des hormones gonadotropes FSH et LH, et de l'œstradiol (E2) qui sont alors similaires, voire supérieurs, à ceux de l'adulte. Cette production précoce d'E2 par l'ovaire est essentielle pour préparer l'organisme à la fonction reproductive. Malgré l'importance de cette activité ovarienne précoce, les mécanismes sous-jacents ont fait l'objet de peu d'études. Précédemment, mon équipe a montré, chez la souris, l'effet de ces niveaux élevés de FSH : de façon paradoxale, ils induisent la production d'E2 dans des petits follicules en croissance sans agir sur leur croissance. Au cours de ma thèse, j'ai mis en évidence, chez la souris, d'autres mécanismes essentiels à la régulation précoce de la synthèse d'E2, impliquant l'hormone anti-Müllérienne (AMH) et les androgènes. J'ai également mis en œuvre une série d'expériences sur des modèles murins permettant d'analyser la vulnérabilité potentielle de l'ovaire au cours de la mini-puberté face à des perturbateurs endocriniens de type dioxine. Ces travaux, visant à identifier les mécanismes régulant l'activité précoce de l'ovaire, devraient permettre *in fine* de mieux comprendre comment se met en place la fonction de reproduction et l'origine de ses dysfonctionnements.

Epigenetics alterations of the murine male reproductive system following gestational exposure to chlordecone

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Chlordecone (CD) is an organochlorine insecticide that has been used in banana crops in the French West Indies. Due to long-term contamination of soils and water, the population is still exposed to CD, which is a known reprotoxic compound. Moreover, exposure to CD in adulthood is associated with an increased risk of prostate cancer (PCa).

In our recent work, we exposed pregnant Swiss mice to CD. The prostates from directly exposed (F1) and nonexposed (F3) male progeny were analyzed. We used immunohistochemistry, RNA-seq and ChIP-seq techniques for the comprehensive analyses of chromatin states in prostate.

We observed an increased prostatic intraepithelial neoplasia phenotype (PIN) in both F1 and F3 generations. We observed that genes implicated in testosterone synthesis were dramatically upregulated in PIN samples, as well as aromatase. Besides, we found a important increase in *Esr2*, *Esrrg* and self-renewal-related gene expression both in F1 and F3 prostates containing PIN.

The alterations in RNA transcription were associated with epigenetic changes. Specifically, we found a global increase in H3K4 trimethylation and a decrease in H3K27 trimethylation in prostate of F1 mice. Some changes in H3K4me3 occupancy in the prostate of CD-exposed mice, detected by ChIP-seq analysis, were observed in the third generation suggesting the transgenerational inheritance.

Our data suggest that developmental exposure to CD leads to epigenetic changes in prostate tissue. The PIN containing samples showed evidence of implication in hormonal pathway and self-renewal gene expression that have the capacity to promote neoplasia in CD-exposed mice.

Mécanismes épigénétiques de l'émergence du facteur de transcription *Sf-1* au cours de la différenciation du lignage gonadotrope hypophysaire

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La différenciation du lignage gonadotrope hypophysaire se déroule durant le développement embryonnaire. Elle se caractérise par l'expression du facteur de transcription *Sf-1,* également appelé *Nr5a1*, qui est le marqueur le plus précoce du lignage gonadotrope. C'est un facteur crucial pour l'émergence du lignage puisqu'il initie notamment l'expression des gènes codant le récepteur de la GnRH et les sous-unités spécifiques des hormones gonadotropes LH et FSH. Ainsi, des anomalies de l'expression de *Sf-1* entraînent un hypogonadisme hypogonadotrope et une stérilité de l'individu. Malgré son importance, les mécanismes épigénétiques régulant son expression durant la spécification gonadotrope sont encore peu connus. Nous avons donc effectué une analyse à haut-débit de l'accessibilité de la chromatine (ATAC-seq) dans trois lignées immortalisées de cellules gonadotropes murines, récapitulant trois stades de la différenciation du lignage gonadotrope. Nous avons identifié dans le locus de *Sf-1* une nouvelle séquence régulatrice de type *enhancer,* transitoirement recrutée durant la spécification du lignage. Par stratégie CRISPR-Cas9, nous avons démontré que cet *enhancer* est nécessaire pour l'émergence du lignage gonadotrope. De plus, nous avons mis en évidence que la fixation du récepteur a des œstrogènes (ERa) sur cet *enhancer* est essentielle pour le remodelage de la chromatine, à la fois au niveau de l'*enhancer* mais aussi du promoteur de *Sf-1*, conduisant ainsi à la transcription du gène. Cette étude a permis d'identifier l'élément régulateur le plus précocement impliqué dans la spécification du lignage gonadotrope. Elle souligne l'importance de ERa dans la régulation épigénétique de ce processus de différenciation.

Présentations – Presentations : Session II
10 novembre – November 10th
15h00 – 16h15

I. **Single sperm genome selection: producing cattle with predetermined paternal genomes**
Luis Aguila, Postdoctoral Fellow, Université de Montréal (Page 23)
15h00 – 15h15

II. **Comprendre la dynamique du profil épigénétique spermatique au cours de la maturation post-testiculaire**
Hong Chen, PhD Student, Université Laval (Page 24)
15h15 – 15h30

III. **Reproductive decline across generations due to paternal MTHFR deficiency and linked to demethylation of young retrotransposons**
Gurbet Karahan, Postdoctoral Fellow, McGill University (Page 25)
15h30 – 15h45

IV. **Analyse fonctionnelle du cil primaire dans l'épididyme : une antenne de signalisation qui médie la voie Hedgehog**
Laura Girardet, PhD Student, Université Laval (Page 26)
15h45 – 16h00

V. **Anatomical and cellular heterogeneity in the mouse oviduct-- its potential roles in reproduction and preimplantation development**
Keerthana Harwalkar, PhD Student, McGill University (Page 27)
16h00 – 16h15

Single sperm genome selection: producing cattle with predetermined paternal genomes

Luis Aguila[1], Remi Labrecque[2], Jacinthe Therrien[1], Patrick Blondin[2], Amélie Tremblay[2], Rafael V. Sampaio[1], Lawrence C. Smith[1].

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Genetic selection programs are consistently limited by independent assortment and crossing over of parental chromosomes during meiosis, causing uncontrollable genomic variability in the offspring. In addition, all selection strategies are performed post-fertilization when the random combination of both parental genomes has already occurred. Thus, it would be greatly advantageous to eliminate meiotic uncertainty by selecting genetically superior gametes prior to fertilization. Here, we report a method to produce cattle offspring with a pre-characterized paternal genome. First, to obtain several identical haploid cells from a single sperm, we produced haploid androgenetic (sperm-derived) embryos by combining oocyte enucleation, intracytoplasmic sperm injection, and embryo culture approaches. These haploid embryos were biopsied for genomic characterization and determination of their genomic value, particularly for SNP markers associated with production traits. Then, we were able to produce biparental embryos by injecting the remaining haploid androgenetic blastomeres into *in vitro* matured bovine oocytes. Finally, such biparental reconstructed embryos were transferred to recipients of which 50% of them successfully led to a pregnancy. Normal embryonic and extra-embryonic development was obsereved in all conceptuses recovered at 100 days of gestation. Therefore, we have identified a novel method to produce cattle with a predetermined paternal genome. Based on their haploid genomic value, we are able to select the best sperm (paternal genome) prior to fertilization and assure that only superior gametes are used for producing the next generation in breeding programs. This research was supported by a grant from NSERC-Canada with L'Alliance Boviteq (LS) and a scholarship by Conicyt-Chile (LA).

Comprendre la dynamique du profil épigénétique spermatique au cours de la maturation post-testiculaire

Hong Chen[1], Marie-Pier Scott-Boyer[1], Arnaud Droit[1], Claude Robert[2], Clémence Belleannée[1]

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Contexte général. Les spermatozoïdes subissent des modifications au cours de leur transit dans l'épididyme durant un processus appelé "maturation post-testiculaire". Bien que des études aient montré que l'épigénome spermatique pouvait être modifié en transitant dans l'épididyme, peu de données existent en ce qui concerne le profile dynamique de méthylation de l'ADN spermatique. De plus, les mécanismes impliqués dans ces changements restent inconnus.

Objectifs et méthodes. Nos objectifs sont de cartographier le profil de méthylation de l'ADN spermatique au cours de la maturation post-testiculaire; et d'identifier les enzymes responsables de ces changements. Les spermatozoïdes provenant du testicule et de trois différents segments de l'épididyme ont été isolés par FACS. Après extraction d'ADN, le profil de méthylation a été identifié par RRBS. D'ailleurs, les enzymes (DNMTs et TETs) potentiellement impliquées dans ces modifications ont été identifiées par immunobuvardage et immunofluorescences. Leurs activités respectives ont été mesurées *in vitro*.

Résultats. Notre approche a permis d'obtenir une pureté d'isolation spermatique d'environ 90% et d'identifier des changements importants de leur profil de méthylation au niveau post-testiculaire (5546 DMS *Caput vs.* Testicule), les changements les plus importants se trouvant entre le testicule et la région proximale de l'épididyme. Les enzymes (DNMT3a et TET1) sont retrouvées dans l'épididyme et les spermatozoïdes, avec une forte activité enzymatique détectée dans le fluide épididymaire.

Conclusion et perspective. L'étude du profil de méthylation spermatique et des mécanismes enzymatiques associés nous a permis de mettre en évidence une contribution potentielle du fluide environnant les spermatozoïdes dans le contrôle de leurs marques épigénétiques.

Reproductive decline across generations due to paternal MTHFR deficiency and linked to demethylation of young retrotransposons

Gurbet Karahan[1,2], Donovan Chan[2], Kenjiro Shirane[3], Sanne Janssen[3], Matthew Lorincz[3] and Jacquetta Trasler[1,2,4,5*]

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Methylenetetrahydrofolate reductase (MTHFR) is a crucial enzyme in one-carbon metabolism with an important role in production of S-adenosyl methionine and methyl groups for cellular processes including DNA methylation. As mouse and human studies have shown that MTHFR deficiency can impact male fertility and sperm DNA methylation, there is the potential for the intergenerational passage of epimutations. Here, our aim was to determine whether the effect of MTHFR deficiency on the testis or sperm DNA methylation was similar or exacerbated from one generation to the next.

While F1 *Mthfr* $^{-/-}$ fathers had minor effects on testis weights and sperm counts with a small increase in abnormal tubules (20%) in the testis; F2 *Mthfr* $^{-/-}$ sons showed a further deterioration in reproductive parameters with more decrease in testis weights, sperm counts and increase in abnormal tubules. Genome-wide DNA methylation analysis revealed that F1 sperm DNA methylation was dramatically affected, with nearly 30,000 CpGs affected, most (99.2%) showing a loss of methylation. Compared to their fathers, >80% of F2 sperm DNA methylation defects overlapped with regions affected in F1 sperm suggesting that there are regions consistently susceptible to MTHFR deficiency. These regions were coincided with genomic loci that late methylated during prenatal germ cell development and highly enriched in young retrotransposons.

The worsening of reproductive parameters in MTHFR-deficient sons versus their fathers suggests that epigenetic defects can accumulate across generations and loss of methylation at retrotransposons could contribute to this effect, findings reminiscent of epigenetic inheritance. (Supported by CIHR)

Analyse fonctionnelle du cil primaire dans l'épididyme : une antenne de signalisation qui médie la voie Hedgehog

Laura Girardet[1], Daniel Cyr[2], Clémence Belleannée[1]

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Introduction. Le cil primaire (CP) est une antenne de signalisation essentielle au développement et contrôle homéostatique des organes via la transduction de la voie Hedgehog (Hh). Leur dysfonction est corrélée à nombre de conséquences cliniques, dont l'infertilité masculine. Dans l'épididyme, les CP se trouvent uniquement à la surface des cellules basales, qui constitueraient un pool de cellules souches épithéliales. Notre objectif est d'étudier la fonction des CP et de la voie Hh dans le contrôle des fonctions épididymaires.

Résultats. Nous avons développé un modèle murin conditionnellement délété (cKO) pour la GTPase ciliaire Arl13b dans les cellules basales. À la suite de la validation de ce modèle, nous avons montré une diminution de la longueur des CP ainsi qu'une altération de la voie Hh par qPCR et microscopie quantitative. Bien que les souris cKO ne présentent pas de problème de fertilité, plusieurs résultats indiquent une dérégulation de l'homéostasie épididymaires : le niveau d'expression de plusieurs marqueurs des cellules basales (Krt5, Krt14, p63) diminue significativement, alors que ceux des cellules principales (AQP9, CFTR) augmentent ; l'aire de la lumière des tubules et l'épaisseur de l'épithélium fluctuent au long de l'épididyme des souris cKO comparées aux contrôles.

Conclusions. Nos résultats suggèrent que l'invalidation du gène ciliaire Arl13b entraine une dérégulation de la voie Hh accompagné d'une altération du maintien homéostatique du tissu. Par la suite, une analyse de Single Cell sera menée pour mieux comprendre ces changements. Ultimement, nos recherches permettront d'explorer le rôle des CP dans les fonctions reproductives et l'infertilité masculine.

Anatomical and cellular heterogeneity in the mouse oviduct-- its potential roles in reproduction and preimplantation development

Keerthana Harwalkar*[,1,2], Matthew J Ford[1], Yojiro Yamanaka*[,1]

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The oviduct/fallopian tube is a tube-like structure that extends from the uterus to the ovary. It is an essential reproductive tissue that provides an environment for internal fertilization and preimplantation development. However, our knowledge of its regional and cellular heterogeneity is still limited. We examined the anatomical complexity of mouse oviducts using modern imaging techniques and fluorescence reporter lines and found that there are basic coiling patterns and turning points in the coiled mouse oviduct can serve as reliable landmarks for luminal morphological regionalities. We identified anatomical structures in the isthmus (ISM) and uterotubal junction (UTJ) that likely play important roles in reproduction. Interestingly, during ovulation, the isthmus was transiently plugged by a thick mucus, keeping the oocytes within the ampulla. Preimplantation embryos travelled along the oviduct and formed a queue within small compartments of the UTJ before uterine entry. Further, the AMP-ISM junction (AIJ) was a unique regionality different from the AMP and ISM in its multi-ciliated cell distribution pattern, luminal morphology, transcriptional factor expression, and acidic mucin secretion. Taken together, our results revealed anatomical and cellular heterogeneity in the mouse oviduct luminal epithelium and suggested functional diversity in each morphologically distinct region.

Conférencière invitée - Invited Speaker

Patricia Ann Hunt

Patricia Hunt is the Meyer Distinguished Professor in the School of Molecular Biosciences at Washington State University, Pullman, WA. She started her research career studying human chromosome abnormalities. Her goal was to understand the high incidence of chromosomally abnormal eggs produced by human females and why the incidence is so strongly impacted by advancing maternal age. She remains fascinated by this complex problem, but the accidental exposure of her mice to bisphenol A (BPA) in 1998 focused her attention on the effects of common environmental contaminants on reproduction. Her current research focuses on chemical mixtures and transgenerational effects of exposure to endocrine disrupting chemicals. Dr. Hunt was named one of the top 50 researchers of the year by *Scientific American* in 2007, was elected to the Washington State Academy of Sciences in 2015 and received the Hartman Award from the Society for the Study of Reproduction in 2018. Her research has been continuously funded by the National Institutes of Health for over 25 years. Dr. Hunt is particularly interested in science communication and is dedicated to ensuring that future trainees not only will be outstanding scientists but easily able to communicate their findings to the general public.

On Tuesday, Dr. Patricia Hunt will give a talk entitled: ***"Maternal exposure and the developing fetus"***.

Présentations – Presentations : Session III
11 novembre – November 11th
09h05 – 10h20

I. **Lack of a robust mitotic checkpoint jeopardises chromosomal stability in mouse embryos**
Adélaïde Allais, PhD student, Université de Montréal (Page 30)
9h05 – 9h20

II. **Function of Ankyrin-repeat and SOCS-box protein 9 (ASB9) in bovine ovarian granulosa cells**
Soma Nostrat Pour, MSc Student, Université de Montréal (Page 31)
9h20 – 9h35

III. **Involvement of Fragile X Related Proteins in the formation of the network of transzonal projections between cumulus cells and the oocyte**
Mathilde Marchais, PhD Student, Université Laval (Page 32)
9h35 – 9h50

IV. **Divergent regulation of dual specificity phosphatases (DUSPs) by fibroblast growth factors**
Lauriane Relav, PhD Student, Université de Montréal (Page 33)
9h50 – 10h05

V. **Kif18a is dispensable in meiosis-I but essential in avoiding aneuploidy in meiosis-II**
Aleksandar Mihajlovic, Postdoctoral Fellow, Université de Montréal (Page 34)
10h05 – 10h20

Lack of a robust mitotic checkpoint jeopardises chromosomal stability in mouse embryos

Adélaïde Allais[1], Greg FitzHarris[1,2]

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In fertility clinics, the timing of cell divisions is highly variable and is an indicator of embryo health. Recently a so-called mitotic clock checkpoint (MitClock) has been described in somatic cells wherein an extended duration of mitosis can cause a subsequent G1/S arrest in somatic cells. But whether this mechanism can operate in the mammalian embryo is unknown. By manipulating the duration of mitosis in two-cell stage mice embryos using an anaphase promoting complex inhibitor, we show that, contrary to somatic cells, preimplantation embryos fail to activate a MitClock after 6-hours prolonged mitoses. However, this mitosis extension leads to premature separation of sister chromatids, known as "cohesion fatigue" (CF), which in turn induces chromosome segregation defects. In contrast, an "extreme" 24-hours-long mitotic arrest causes a potent cell cycle arrest in the subsequent interphase. This arrest is partially reversed by simultaneous addition of a spindle poison (monastrol). Moreover, extreme mitosis prolongation causes an increase of DNA damage measured using γH2AX immunoreactivity. Strikingly, mitosis prolongation did not increase DNA damage in the presence of monastrol, which we also show prevents CF, together implying that extreme M-phase prolongation activates a checkpoint based on DNA damage downstream of sister-chromatid individualisation. Our data thus show the absence of a robust MitClock in embryos, which paired with the apparent propensity of embryos to exhibit CF, likely contributes to the high level of mosaic aneuploidy in embryos. Only extreme M-phase extensions elicit a cell cycle arrest in embryos, in a mechanism that likely involves DNA damage.

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Function of Ankyrin-repeat and SOCS-box protein 9 (ASB9) in bovine ovarian granulosa cells

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Background: Ankyrin-repeat and SOCS-box protein 9 (ASB9) is a member of the large SOCS-box containing proteins family. We previously reported *ASB9* as a differentially expressed gene in granulosa cells (GC) of ovulatory follicles following hCG injection. The current study aimed to further investigate the role of ASB9 in GC and decipher ASB9 mechanism of action in GC. **Methods**: An *in vivo* model of GC was obtained from follicles at different developmental stages: small follicles (SF), dominant follicles (DF) and ovulatory follicles (OF) to analyze ASB9 regulation and its potential targets PAR1, TNFAIP6, and TAOK. Additionally, an *in vitro* model of cultured GC was used along with the CRISPR/Cas9 approach, to inhibit ASB9 in GC. **Results**: Western blot analyses demonstrated ASB9 induction in GC by hCG from 12h post-hCG through 24h. RT-qPCR analyses using *in vivo* samples showed greater expression of *PAR*1 in DF as compared to OF while *TNFAIP6* and *TAOK1* are induced in OF by hCG. Inhibition of ASB9 via CRISPR/Cas9 was confirmed by RT-qPCR and resulted in a significant increase in *PAR1*, *PCNA*, *CCND2* and *CCNE2*, significant decrease in *TAOK1*, *TNFAIP6* and *CASP3* steady-state mRNA expression, and no effect on *CCNA2*. Results show an increase in GC proliferation and reduction in Caspase3/7 activity following ASB9 inhibition using CRISPR/Cas9. Western blot analyses showed an increase in ERK1/2 phosphorylation level following ASB9 inhibition. **Conclusion**: Taken together, our results provide strong evidence that ASB9 could be a regulator of GC proliferation and function contributing to GC differentiation into luteal cells.

Involvement of Fragile X Related Proteins in the formation of the network of transzonal projections between cumulus cells and the oocyte

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Communication between the oocyte and somatic cells is essential for successful ovarian folliculogenesis. We previously have shown that messenger RNA expressed in the cumulus cells can transit across the transzonal projections (TZPs) to be transferred to the oocyte. We hypothesize that proteins from the Fragile X Related (FXRP) family are involved in establishing the TZP network and transporting mRNAs across them. The FXRP protein family is composed of FMRP, FXR1P and FXR2P. Although in human, a reduced expression of FMRP is associated with prevalence of premature ovarian failure, the mouse knockout for FMRP does not show a reproductive phenotype.

Our preliminary data shows a compensatory mechanism by FXR2P in absence of FMRP, with higher expression of FXR2P in the compartments and stages where FMRP was abundant. Oocytes from *Fmr1* null mice still show a present but abnormal TZP network with more abundant but shorter and straighter TZPs. While *Fmr1*$^{-/-}$/*Fxr2*$^{-/-}$ females are infertile, *Fmr1*$^{+/-}$/*Fxr2*$^{+/-}$ and *Fmr1*$^{+/-}$/*Fxr2*$^{-/-}$ genotypes display a reduced reproductive lifespan. Moreover *Fmr1*$^{-/-}$/*Fxr2*$^{-/-}$ males and females mice exhibit facial abnormalities and *Fmr1*$^{-/-}$/*Fxr2*$^{-/-}$ and *Fmr1*$^{-/-}$/*Fxr2*$^{+/-}$ show behavioral abnormalities. Allele inactivation of any of the genes lead to macroorchidism in males. Ovaries and oocytes from the different genotypes are being characterized. To date, our results strongly support the involvement of FXRP proteins in the formation of the TZP network which is essential to produce a good quality egg. Ultimately, the roles of FXRP proteins could highlight a new key physiological process for folliculogenesis and oogenesis.

This project is supported by a grant from the Natural Sciences and Engineering Research Council of Canada.

Divergent regulation of dual specificity phosphatases (DUSPs) by fibroblast growth factors (FGFs) in ovine granulosa cells

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Numerous factors control ovarian folliculogenesis through the regulation of granulosa cell function. Fibroblast growth factors (FGF) are one of those and mainly activate the mitogen-activated protein kinase (MAPK) signalling pathway to promote cell proliferation and function. Dual-specificity phosphatases (DUSPs) regulate the magnitude and duration of phosphorylation of second messenger signals, including that of MAPKs. There are 23 DUSPs genes in mammals, and we have previously reported at RQR that 16 of these DUSPs are expressed in ovine granulosa cells. Our hypothesis is that regulation of DUSPs may alter MAPK signalling in granulosa cells. The aim of the present study was to describe the regulation and potential action of DUSPs in sheep granulosa cells. We used a well characterized serum-free culture model to determine the effect of two divergent FGFs, FGF2 and FGF18, on DUSP expression. Treatment with FGF2, but not FGF18, increased *DUSP1*, *DUSP5*, and *DUSP6* mRNA levels at 1h, all the time points (1h, 2h, 4h, 8h), and 8h respectively. Western blot analysis demonstrated that DUSP1 protein abundance was significantly increased at 3h, and DUSP6 at 8h, 12h, 24h. We then used the DUSP1 and DUSP6 selective inhibitor BCI (10 µM) to determine the role of these DUSPs. Pre-treatment with BCI abolished FGF2-induced MAPK3/1 phosphorylation, but increased JNK phosphorylation.

We conclude that DUSP1 and DUSP6 are important regulatory components of the MAPK pathway in sheep granulosa cells.

Kif18a is dispensable in meiosis-I but essential in avoiding aneuploidy in meiosis-II

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Chromosome segregation fidelity during the two meiotic divisions is essential for the production of high-quality gametes and heavily relies on molecular motor proteins responsible for spindle assembly and chromosome movement. One such protein implicated in regulation of spindle length and chromosome alignment in mitotic cells is Kif18a, however, its role in meiosis has not been examined. Here, we employed Cre-loxP technology and crossed Zp3-Cre$^{Tg/Tg}$ transgenic mouse line with Kif18a-GFP$^{flox/flox}$ reporter mice to conditionally knock-out(KO) *Kif18a* gene in oocytes and investigate its role in female meiosis. We show that Kif18a protein normally becomes localized at the spindle late in meiosis-I(MI) and progressively accumulates by meiosis-II(MII). Live-imaging revealed that Kif18a-deficient oocytes were able to successfully complete MI with no observable chromosome segregation defects leading to conclusion that Kif18a is dispensable for meiosis-I chromosome segregation. In contrast, Kif18a-deficient oocytes exhibited ~4-fold increase in chromosome congression failure in MII(22% in KO vs. 5% in control). Among these, more than half of the observed oocytes(~13%) harboured severely misaligned sister chromosome pairs that missegregated following parthenogenetic egg activation. Thus, Kif18a deficiency leads to aneuploidy in MII. In addition, we observed ~4-fold increase in lagging chromosome frequency in Kif18a-KO eggs during anaphase-II(61% in KO vs. 14% in control), that could additionally cause aneuploidy. Thus, multiple chromosome segregation defects arise in the absence of Kif18a during meiosis-II. Taken together, our results reveal an essential role for Kif18a in chromosome segregation fidelity during meiosis-II, but notably establish meiosis-I as potentially unique in having dispensed with Kif18-a dependent chromosome alignment.

Conférencier invité - Invited Speaker

Thomas E. Spencer

Tom Spencer is a Curators Distinguished Professor in the Division of Animal Sciences and Department of Obstetrics, Gynecology and Women's Health at the University of Missouri in Columbia, Missouri. The long-term goal of his research program is to discover and understand key physiological and genetic mechanisms regulating development and function of the uterus and placenta and translate that knowledge to improvement of fertility in domestic animals and humans. His research in reproductive and developmental biology utilizes a number of different animal models (sheep, beef cattle, dairy cattle, mice) as well as human tissues. Broadly, current discovery foci of the program include understanding: (1) cellular and molecular mechanisms regulating gland development and function in the uterus using genetically engineered mice and sheep; (2) genetic pathways regulating fertility in beef cattle, dairy cattle, mice and humans; and (3) physiological pathways regulating pregnancy recognition and establishment using cattle, sheep and mice. Spencer has a vigorous research and graduate education program that has earned several awards including: Society for the Study of Reproduction Research Award; American Society of Animal Science Physiology and Endocrinology Award; and Fellow of the American Association for the Advancement of Science. In 2019, he was elected as a member of the National Academy of Sciences. He has published over 300 scientific articles, reviews and book chapters. His research is funded by active grants from the NIH (R01 and R21), USDA NIFA (Challenge, Dual Purpose, and Foundational), and Science Foundation Ireland.

On Wednesday, Dr. Thomas Spencer will give a talk entitled: ***"Insights into the Biological Roles of Uterine Glands in Pregnancy Establishment"***.

Session d'affiches – Poster Session

1. Targeted disruption of *Lats1* and *Lats2* in Sertoli cells impairs the development of the testis. *Nour Abou Nader, PhD Student, Université de Montréal (Page 40)*

2. Role of specific Akt Isoforms during Decidualization Using A PR-Cre Mouse Model. *Pascal Adam, PhD Student, Université du Québec à Trois- Rivières (Page 41)*

3. Gonadotropin-releasing hormone signaling via chimeric receptors. *Carlos Agustin Alonso, Postdoctoral Fellow, McGill University (Page 42)*

4. Role of Hedgehog pathway in the control of fluid reabsorption in the efferent ductules. *Céline Augière, Postdoctoral Fellow, Université Laval (Page 43)*

5. In-utero exposure to sterile inflammation is associated with altered neurodevelopment. *Marie-Ève Brien, PhD Student, Université de Montréal (Page 44)*

6. Development of a SNP Chip for caribou/reindeer (*Rangifer tarandus*). *Alexandra Carrier, PhD Student, Université Laval (Page 45)*

7. Abnormalities in the sperm DNA methylome both pre- and post-treatment in men with Hodgkin's disease and testicular cancer. *Donovan Chan, Research Professional, McGill University (Page 46)*

8. Cellular factors influencing aggregate morphology. *Matthew Chang, BSc Student, McGill University (Page 47)*

9. Differential Expression of Alternatively Spliced Disabled-1 (DAB1) Isoform in Granulosa Cells. *Marianne Descarreaux, MSc Student, Université de Montréal (Page 48)*

10. Dysregulation of FOXO3a-dependent transcriptional programs and cellular functions during *Toxoplasma gondii* Infection. *Andrés Felipe Díez Mejía, PhD Student, INRS-Institut Armand Frappier (Page 49)*

11. Identification du mécanisme d'action moléculaire du récepteur nucléaire orphelin LRH-1 en réponse au signal ovulatoire dans les cellules de la granulosa. *Florence Gagnon, MSc Student, Université de Sherbrooke (Page 50)*

12. HMGB1 endogenous mediator of inflammation at the maternal fetal interface. *Virginie Gaudreault, PhD Student, Université de Montréal (Page 51)*

13. Hypométhylation spécifique de gènes à empreinte en lien avec l'âge de la donneuse d'ovocytes et les conditions de culture in vitro d'embryons bovins au jour 7. *Simon Lafontaine, PhD Student, Université Laval (Page 52)*

14. Implication of mutated DNMT3A in the Pathogenesis of Tatton-Brown-Rahman Syndrome. *Alexandra Langford, MSc Student, Université de Montréal (Page 53)*

15. Characterization of the Ser/Thr phosphatase PPEF1 in spermatozoa. *Camille Lavoie-Ouellet, MSc Student, Université Laval (Page 54)*

16. Investigating Heritable Epigenetic Dysregulations in Promoter Regions Following a Temporary Inhibition of Dnmt1 in Mouse Embryonic Stem Cells. *Anthony Lemieux, MSc Student, Université de Montréal (Page 55)*

17. Characterization of micro-peritoneal metastasis in the mouse ovarian cancer model. *YuQi Li, MSc Student, McGill University (Page 56)*

18. Transcription of a novel inhibin B co-receptor is regulated by steroidogenic factor 1. *Claire Lin, MSc Student, McGill University (Page 57)*

19. Mitochondrial network organisation in cumulus cells. *Amel Lounas, PhD Student, Université Laval (Page 58)*

20. Early Detection of Abnormal Placental DNA Methylation and Gene Expression Associated with Assisted Reproduction: Differential Effects of Folic Acid Supplementation. *Josée Martel, Research Profesionnal, McGill University (Page 59)*

21. Réorganisation des sites de liaisons de LRH1 suite au signal ovulatoire chez les cellules de la granulosa murine*. Fanny Morin, MSc Student, Université de Sherbrooke (Page 60)*

22. Rôle immunomodulateur de TGFβ1 sur les voies inflammatoires induites par l'INFγ et le GM-CSF dans les trophoblastes et les macrophages humains. *Khiat Nihad, PhD Student, Université du Québec à Trois-Rivières (Page 61)*

23. Development of a highly sensitive enzyme-linked immunosorbent assay (ELISA) for measurement of follicle-stimulating hormone (FSH) in serum, plasma, and whole blood in mice. *Luisina Ongaro, Research Professional, McGill University (Page 62)*

24. Cytokinesis fails to scale to cell size in the early mouse embryo. *Lia Paim, PhD Student, Université de Montréal (Page 63)*

25. Development of a new method for pig karyotyping. *William Poisson*, *MSc Student, Université Laval (Page 64)*

26. Rôle régulateur de l'Oncostatine M (OSM) dans les trophoblastes en présence de signaux pro-inflammatoires. *Marion Ravelojaona, PhD Student, Université du Québec à Trois-Rivières (Page 65)*

27. Les convulsions fébriles sont associées à des altérations de l'expression de gènes dans le placenta à terme. *Morgane Robles, Postdoctoral Fellow, INRS-Institut Armand Frappier (Page 66).*

28. XIST DMR methylation levels and knockdown in bovine somatic cells and SCNT-derived embryos. *Rafael Sampaio, Postdoctoral Fellow, Université de Montréal (Page 67)*

29. ZEB1 inhibits *Lhb* transcription by blocking the stimulatory actions of EGR1. *Hailey Schultz, MSc Student, McGill University (Page 68)*

30. Gonad-Specific Knockout of Steroidogenic Factor 1 (SF-1) leads to impaired fertility in male mice. *Olivia Smith, PhD Student, Université de Montréal (Page 69)*

Targeted disruption of *Lats1* and *Lats2* in Sertoli cells impairs the development of the testis

Nour Abou Nader[1], Bérengère Deffrennes[1], Derek Boerboom[1], Gustavo Zamberlam[1], Alexandre Boyer[1]

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Sertoli cells occupy a unique place in testis development, playing major roles in the process of sex determination and the ensuing differentiation of the testis cell lineages. It is therefore important to have a better understanding of the signaling pathways involved in the regulation of these processes. Hippo pathway is a signaling pathway with well-established roles in cell proliferation and differentiation mechanisms, however its role in the developing Sertoli cells still needs to be elucidated. To study the involvement of the Hippo pathway in the development of the testes, we generated a mouse model (*Lats1*$^{flox/flox}$*;Lats2*$^{flox/flox}$*;Amh*-Cre) in which *Lats1* and *Lats2* were conditionally deleted in Sertoli cells. Preliminary characterization of this model demonstrate a decrease in testicular weight in the mutant animals. Histopathologic evaluation reveal that LATS1/2 are necessary for the maintenance of spermatogenesis, and that their loss leads to the progressive degeneration of the testis cords including the loss of polarity of some SOX9-positive Sertoli cells. Our preliminary results confirm that the Hippo signaling pathway is required to maintain the function of the Sertoli cells. This model needs further investigation to determine the mechanisms of action of the Hippo signaling pathway in Sertoli cells.

Role of specific Akt Isoforms during Decidualization Using A PR-Cre Mouse Model

Pascal Adam[1], François Fabi[1], Laurence Tardif[1], Sophie Parent[1], Eric Asselin[1]

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Infertility is a rising problem for North American couples trying to conceive. Successful implantation is primordial and necessitates a receptive endometrium dependent of decidualization. Driven by progesterone and cAMP, endometrial stromal cells undergo important phenotypical changes during this process involving many signaling factors in proliferation and apoptosis such as PI3K/Akt pathway. Three Akt isoforms have been identified and are recognized to have distinct physiological and pathological roles in different processes. Previously, *in vitro* experiments demonstrated that expression and activity of specific Akt isoforms are downregulated during decidualization, but little is known about their implication in cell survival, apoptosis and glycogen synthesis. We hypothesize each Akt isoform have specific roles and are differently regulated throughout the decidualization process. Therefore, we developed a unique endometrial-targeted mouse model with simple and combined KO of each Akt isoforms with the PR-Cre mouse model. Using artificial decidualization during pseudopregnancy, the implication of PI3K/Akt pathway in this process was evaluated. Our results suggest subfertility phenotypes such as variation of the average mouse litter number and absence of decidualization in Akt1-2 KO, showing redundant roles between those isoforms (Akt1 KO or Akt2 KO mouse showed a more regular phenotype). Also, preliminary results suggest that p70S6K activity is regulated by Akt2 and IκBα by Akt3 during a non-gestational, progesterone driven context in the endometrium. Further experiments will allow us to understand the precise signaling mechanisms by which this pathway is regulated by each isoform during decidualization and could possibly lead to better develop strategies to reduce fertility issues.

Gonadotropin-releasing hormone signaling via chimeric receptors

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Reproduction depends on signaling by gonadotropin-releasing hormone (GnRH) in pituitary gonadotropes. Gonadotropes of all vertebrate species express orthologs of the GnRH receptor (GnRHR), a G protein-coupled receptor (GPCR). The mammalian GnRHR lacks an intracellular C-terminus (Ctail), present in most GPCRs. The significance of this specialization is presently unknown. We previously showed that mice engineered to express a chimera of the murine GnRHR fused to the chicken (*Gallus gallus*) GnRHR Ctail exhibit decreased follicle-stimulating hormone production, a blunted luteinizing hormone surge, and reduced litter sizes. Pituitary cell cultures from these animals show impaired GnRH-stimulated Ca^{2+} mobilization, which appears to result from reduced coupling of Gq to the receptor. Collectively, these data suggested that that loss of the Ctail may have enhanced G protein coupling to the mammalian GnRHR, leading to increased gonadotropin production and fertility. However, the sequence of the GnRHR Ctail is highly divergent across non-mammalian vertebrates. We therefore investigated whether addition of the Ctail from another species, *Xenopus laevis*, to the murine GnRHR similarly affected signaling. As observed previously, GnRH-induced Ca^{2+} mobilization was impaired in HEK293 cells transfected with the murine-chicken GnRHR receptor relative to wild-type murine GnRHR. In contrast, cells expressing the murine-frog GnRHR chimera exhibited Ca^{2+} increases similar to the wild-type receptor. GnRH-stimulated increases in ERK1/2 phosphorylation was similar downstream of all three receptors and was dependent on novel (Ca^{2+} independent) protein kinase C isoforms. In conclusion, the addition of C-tails of different vertebrate GnRHRs to the murine GnRHR differentially influences downstream signaling.

Role of Hedgehog pathway in the control of fluid reabsorption in the efferent ductules

Augière Céline[1], Bernet Agathe[1], Joly Beauparent Charles[1], Droit Arnaud[1], Soulet Denis[1], Hess Rex[2], Belleannée Clémence[1]

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Efferent ductules are small tubules connecting the testis with the head of epididymis. These tubules contribute to maintain a proper ratio sperm/fluid through mechanisms of fluid absorption, whose impairment results in male infertility. The efferent ductules encompass two types of cells: ciliated cells that expose motile cilia stirring the sperm fluid, and monociliated cells known to play a role in fluid reabsorption. Our recent discoveries indicated that monociliated cells expose at their surface a unique atypical primary cilia organelle. While in other model systems primary cilia are sensory cilia involved in the control of organ development and homeostasis by transducing hedghehog signaling pathway, nothing is known as regards to these sensory primary cilia in the efferent ductules. We developped a conditional KO (cKO) to disturb Hedgehog signalling in efferent ductules in order to decipher the role of this pathway in adult male reproductive physiology. Knock out mice display oversized efferent ductules with enlarged dilated tubules compared to control littermates, a phenotype that is associated with the alteration of ion channel-transporters expression according to our RNAseq data. Furthermore, we demonstrated that CFTR, a chloride channel whose impairment triggers male infertility, displays an ectopic localization on cilia in cKO mice. This underscores the unexpected contribution of the hedgehog signalling pathway in the regulation of CFTR and other ion channels, and in the maintenance of a proper hydric balance in the efferent ductules. Our results shed light on novel cellular and molecular mechanisms that monitor the homeostatic status of the male reproductive system.

In-utero exposure to sterile inflammation is associated with altered neurodevelopment

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Prenatal inflammation alters placental function having a negative impact on fetal development and is associated with an increased risk of neurodevelopmental disorders. Infectious stimuli are commonly used in animal model; however, infections are often undetectable during pregnancy whilst inflammation is observed. We developed an animal model of prenatal non-infectious inflammation, induced by uric acid crystals, leading to placental inflammation and fetal growth restriction (FGR) (Brien et al., 2017). Our objective was to further study the long-term effect of prenatal exposure to non-infectious inflammation on the developing brain. METHODS: The impact of in utero inflammation was determined on the brain from gestational day 22 (GD22) to postnatal day 50 (PND50) using our published model. Immunohistological analysis were performed for micro/astro-glial activation and neuronal precursors. Behavioral testing was performed to evaluate motor and cognitive function. We also investigated the therapeutic potential of targeting the interleukin (IL)-1 system. RESULTS: Prenatal exposure to inflammation led to microgliosis in the corpus callosum at PND7 as well as in the hippocampus (CA3 and DG) at PND7/21. Astrogliosis was observed in the white matter (both cc and cg), in the motor cortex and hippocampus at PND7. Decreased number of neuronal precursors was also observed in the DG at later developmental stages. IL-1 receptor antagonist protected against most of the structural. Motor functions were also affected by prenatal uric acid exposure. CONCLUSION: Prenatal exposure to non-pathogenic inflammation has important negative impact on brain development. Futures studies will investigate prenatal anti-inflammatory intervention to protect the developing brain.

Development of a SNP Chip for caribou/reindeer (*Rangifer tarandus*)

Alexandra Carrier[1,2,3], Julien Prunier[4], William Poisson[1,2,3], Laurence Auger[5], Yohan Harton[5], Isabelle Gilbert[1,2,3], Juha Kantanen[6], Marco Musiani[7], Steeve D. Côté[5], Vicky Albert[8], Vincent Bourret[8], Arnaud Droit[4], Claude Robert[1,2,3]

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This project stems from a collaboration between the Ministère des Forêts, de la Faune et des Parcs du Québec (MFFP) and Laval University to develop a genotyping array for caribou. Genomic capacities have been voiced by MFFP to meet two specific needs: monitoring caribou populations using genomics; providing a tool for population assignment to support protection efforts of specific caribou ecotypes. An additional objective was to design a SNP array functional for *Rangifer tarandus* populations across the entire species geographic distribution. A catalog of Single Nucleotide Polymorphisms (SNP) was established using different sequencing strategies on individuals mainly from genetic regions of Québec and Labrador previously identified but caribous from other regions of Canada, Alaska, Russia, Finland, Norway and Greenland were also included. Overall, 20 individuals were sequenced in short reads with a 30X coverage, 192 individuals were sequenced with a 5X coverage and 700 were submitted to genotyping by sequencing (GBS). From this, a catalog of 30 M SNPs was assembled. A panel of 50,000 SNPs was assembled evenly spaced across the entire reference genome in order to estimate metrics associated with genomic diversity. These SNPs were selected from the intersect between populations of Quebec-Labrador and outgroups from the rest of the species distribution. An additional 10,000 SNPs were selected for population assignment purposes, based on supervised and unsupervised clustering. Finally, 2,500 SNPs associated with behavioural patterns have been included. So far, the Caribou SNP Chip has been designed by Illumina and previous samples have been submitted to genotyping. This project is supported by a partnership grant from Génome Canada and Génome Québec.

Abnormalities in the sperm DNA methylome both pre- and post-treatment in men with Hodgkin's disease and testicular cancer

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Although combination chemotherapy has contributed to increased survival from Hodgkin's disease (HD) and testicular cancer (TC), questions about sperm quality after treatments have arisen. Studies have shown DNA damage post-treatment, however, the sperm epigenome has received little attention. Our objectives were to determine the impact of HD and TC, as well as their treatments, on sperm DNA methylation. Sperm were collected from community controls (CC) and men with HD or TC (before and after chemotherapy). DNA methylation was assessed using genome-wide and locus-specific approaches. Before treatments, using 450K arrays, a subset of probes distinguished sperm from CC, HD and TC subjects.Comparing sperm methylation between HD or TC patients versus CC, twice as many sites were affected in TC than HD, both demonstrating mostly hypomethylation. In TC patients, the promoter region of *GDF2* contained a large region of differential methylation. To assess alterations in DNA methylation over time/post-treatment, serial samples from individual patients were compared. With 450K arrays, following chemotherapy, patients showed increased alterations in DNA methylation, up to several years post-treatment, when compared to CC. Similarly, using a high-resolution human sperm-specific assay, that includes assessment of environmentally-sensitive (dynamic) sites, demonstrated altered DNA methylation in patients post-treatment and suggested preferential susceptibility of dynamic CpGs.Distinct sperm DNA methylation signatures were present pre-treatment in men with HD and TC. Epigenetic defects in some cancer survivors are present up to two years post-treatment. Abnormalities in the sperm epigenome both pre- and post-chemotherapy may have detrimental effects on future health. Funded by CIHR.

Cellular factors influencing aggregate morphology

Matthew Chang[1, 2], Yojiro Yamanaka[1,3]

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During embryogenesis, mammalian embryos shape themselves through compaction, polarization, and tubulogenesis. The epithelium is the fundamental tissue structure driving these morphogenetic events; however, the regulation is not fully understood. We propose that how individual cells interact with others through cell-cell contacts dictates the resulting aggregate shape and cell allocation, leading to the complex 3D structures of tissues and embryos. Therefore, understanding the mechanisms regulating cell-cell contact and biophysical properties of individual epithelial cells is critical. COLO205, an ascites-derived colorectal adenocarcinoma line, is a great model to study relationships between cell-cell contact regulation and 3D aggregate morphology. Although COLO205 cells express fully functional E-cadherin complexes, they do not form cell-cell contacts efficiently until 3-5 days post-passage.To identify the factors influencing cell-cell contact and aggregate morphology, we cultured COLO205 in different conditions. Interestingly, the culture media impacted the shape of cell aggregates. COLO205 cultured in DMEM/F12 formed round and compacted aggregates, while being loose and bumpy in RPMI. Although both aggregates formed cell-cell contacts, distribution patterns of E-cadherin complex members differed. This aggregation formation appeared to be cell-density dependent, and supplementation of ECM components decreased the time for aggregation. Finally, we performed inhibitor screenings and identified Src family kinases as potential candidates in regulating cell shape, with similar aggregate shapes in both media. Therefore, we hypothesize that there exist several conditions to initiate aggregation, but likely though different mechanisms.

Our study will provide molecular mechanisms responsible for controlling epithelial cell morphology and their regulation during development and in diseases like cancer.

Differential Expression of Alternatively Spliced Disabled-1 (DAB1) Isoform in Granulosa Cells

Marianne Descarreaux[1], Kalidou Ndiaye[1], Jacques G. Lussier[1]

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Disabled-1 (DAB1) is a cytosolic adaptor protein crucial for signaling of extracellular protein Reelin following its binding to the receptor LRP8, also known as ApoER2. Transduction of the signal by DAB1 is particularly important during brain cortex development in vertebrates. We previously demonstrated that LRP8 is strongly expressed in granulosa cells (GC) of the dominant or preovulatory follicle, while Reelin is expressed within the theca layer. Our results suggest that the signaling pathway Reelin/LRP8/DAB1 contributes to the growth of the dominant or preovulatory follicle. The objective is to study the expression and function of DAB1 in the GC of the bovine ovarian follicle.

We compared the expression of *DAB1* mRNA expressed in GC and bovine cerebral cortex. Expression and regulation of *DAB1* were studied by RT-qPCR in GC obtained from small follicles (SF: 2-4 mm), dominant follicles at day 5 of the estrus cycle (DF), ovulatory follicles 24 hours following injection of hCG (OF) and corpus luteum at day 5 of the estrus cycle.

We found that the DAB1 isoform expressed in GC is different from the one expressed in the brain and it is the first characterization of this DAB1 isoform in mammalian GC. Analysis by RT-qPCR showed that *DAB1* is expressed at greater amounts in DF compared to SF, OF and CL ($P<0.0001$). Our results will contribute to a better understanding of LRP8 signaling in GC and its involvement in the mechanisms regulating fertility in mammals.

Dysregulation of FOXO3a-dependent transcriptional programs and cellular functions during *Toxoplasma gondii* Infection

Andrés Felipe Díez[1], Louis-Philippe Leroux[1], Sophie Chagneau[1], Cathy Vaillancourt[1], Maritza Jaramillo[1].

[1]Dysregulation of FOXO3a-dependent transcriptional programs and cellular functions during *Toxoplasma gondii* Infection

Forkhead box O3a (FOXO3a) is a transcription factor that mediates essential cellular processes that include proliferation, resistance to oxidative stress, inflammation, apoptosis, and endometrial decidualization. Accordingly, reduced FOXO3a levels in the uterus are associated with spontaneous abortion. *Toxoplasma gondii* (*T. gondii*) is the etiological agent of congenital toxoplasmosis, a parasitic infection that can lead to miscarriage and premature births; however, the molecular underpinnings of disease pathogenesis remain poorly defined. We demonstrated that *T. gondii* represses translation of *Foxo3a* mRNA and thereby reduces FOXO3a protein synthesis in the host cell. This phenotype was confirmed in several cell types present in the villous stroma, namely trophoblasts, macrophages, and fibroblasts. Hence, we postulate that alteration of transcriptional programs downstream of FOXO3a contribute to placental dysfunction during congenital toxoplasmosis. To address this, we initially focused on fibroblasts as they are the most permissive cell type to *T. gondii* infection in the villous stroma. Subcellular fractionation experiments combined with image flow cytometry approaches showed a reduction in nuclear FOXO3a levels in a human foreskin fibroblast (HFF) cell line infected with *T. gondii*. In line with these data, mRNA and protein expression of FOXO3a transcriptional target genes p130 (cell cycle), Beclin-1 (autophagy), and catalase (oxidative stress) were reduced in *T. gondii*-infected HFF. Ongoing experiments using chemical compounds and forward-genetics approaches to modulate FOXO3a expression, localization and/or activity in fibroblasts and other placental populations will provide further insight on the role of dysregulated FOXO3a-dependent transcriptional programs in placental pathology associated with congenital toxoplasmosis.

Identification du mécanisme d'action moléculaire du récepteur nucléaire orphelin LRH-1 en réponse au signal ovulatoire dans les cellules de la granulosa

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L'infertilité, définie par l'incapacité d'établir une grossesse après un an de rapports sexuels non protégés, affecte 12-15% des couples en âge de procréer représentant 186 millions d'individus à l'échelle mondiale. Environ 40% des cas reliés à l'infertilité féminine ont été attribués à un dysfonctionnement ovarien, mais les mécanismes moléculaires coordonnant les processus physiologiques d'ovulation et de lutéinisation lors du cycle ovarien sont encore méconnus. Dans un modèle murin, il a été démontré que le récepteur nucléaire orphelin Liver receptor homolog 1 (LRH-1) est indispensable à tous les stades du cycle ovarien. Les travaux antérieurs des professeurs Murphy et Gévry ont confirmé qu'à la suite du signal hormonal initiant l'ovulation et la lutéinisation, les cellules de la granulosa subissent un remodelage important de la chromatine. Ce remaniement coïncide avec la reprogrammation d'une partie des sites d'action dans le génome (cistrome) de LRH-1. Notre hypothèse est que la modification des sites de liaison à l'ADN de LRH-1 est causée par un changement des cofacteurs partenaires de LRH-1 à la suite du signal ovulatoire. Le but principal de nos recherches est d'explorer les complexes protéiques associés à LRH-1 dans l'établissement d'un patron d'expression génique spécifique à l'ovulation en exploitant les technologies émergentes en protéomique. L'approche « Proximity Dependent Biotin Identification (BioID) » sera utilisée pour étudier l'interactome de LRH-1 avant et après le signal ovulatoire dans les cellules de la granulosa murines immortalisées (GRMO2). Cette étude permettra de discerner les mécanismes moléculaires essentiels à LRH-1 en réponse au signal ovulatoire.

HMGB1 endogenous mediator of inflammation at the maternal fetal interface

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Placental inflammation has been linked to pregnancy complications. Damaged-associated molecular pattern (DAMP), were demonstrated to induce inflammation and were associated with placental inflammation and dysfunction. Levels of one of these DAMPs, High mobility group box 1 (HMGB1), was demonstrated to contribute to the inflammatory response in PE. HMGB1 is a nuclear protein that can accumulates in the cytoplasm and be secreated in the extracellular space. HMGB1 exert pro-inflammatory activity by mainly binding to specific receptors, RAGE, TLR4, TLR9 and others. The involvement of HMGB1 and its receptors in pregnancy pathologies is not fully understood.

Methods: Placenta and foetal membranes from either normal pregnancy, PE or post-partum preeclampsia (PPPE) was used to determine the distribution of HMGB1 and its receptors (RAGE and TLR4). Term placental explants from normal pregnancy treated with exogenous HMGB1 isoforms was used to determine the pro-inflammatory abilities of HMGB1 and effects on placental function.

Results: In placentas from pregnancies with PE and PPPE, increased percentage of trophoblast with cytoplasmic distribution of HMGB1 was observed as compared to the classic nuclear localisation in normal pregnancies. In fetal membranes, HMGB1 is highly expresses in chorion with major cytoplasmic localisation. HMGB1-disulfide lead to increase secretion of pro-inflammatory cytokines in placental explants (IL-1b, IL-6 and MCP1).

Conclusion: We demonstrated changes in the localization of HMGB1 in placenta and fetal membranes of pregnancies complicated with PE or PPPE. We also showed that HMGB1-disulfide isoform induced inflammatory cytokines and inflammatory pathway activation suggesting the role of this DAMP in placental inflammation.

Hypométhylation spécifique de gènes à empreinte en lien avec l'âge de la donneuse d'ovocytes et les conditions de culture *in vitro* d'embryons bovins au jour 7

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La production d'embryons bovins par maturation et fécondation in vitro est un outil important de la révolution génomique de l'industrie laitière. Nous avons formulé l'hypothèse qu'une partie des patrons de méthylation de l'ADN est acquise dans la dernière partie de la folliculogénèse et pourrait être influencée par l'environnement créé pour produire ces ovocytes. Ces différences pourraient ne pas être effacées durant la première semaine de culture ou potentiellement être sensibles aux conditions durant cette période. Un groupe témoin (in vivo) constitué de 10 embryons (jour 7) provenant de vaches superovulées et inséminées artificiellement fut comparé à des embryons provenant d'ovocytes dérivés d'un protocole de stimulation ovarienne sur des vaches adultes ou des génisses prépubères (10 embryons), récoltés par ponction transvaginale et mis en culture avec (10 embryons) ou sans sérum fœtal bovin (10 embryons). L'ADN de chaque embryon fut traité au bisulfite de sodium pour révéler les marques de méthylation de 4 gènes (PLAGL1, KCNQ1, SNRPN et IGF2R) par pyroséquençage. Pour chaque gène, nous avons observé une méthylation globalement moins élevée ainsi qu'une plus grande variabilité chez les trois groupes d'embryons produits in vitro. 2 embryons dérivées d'ovocytes de donneuses prépubères et 3 embryons mis en culture avec du FBS présente une hypométhylation à deux gène ou plus. Ces résultats concordent avec la perturbation épigénétique observée précédemment dans la littérature et son lien avec le syndrome du gros veau. Ces travaux permettront d'identifier les sites de contrôle de l'empreinte parentale sensibles aux conditions de production in vitro.

Implication of mutated DNMT3A in the Pathogenesis of Tatton-Brown-Rahman Syndrome

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Tatton-Brown-Rahman Syndrome (TBRS) is a rare genetic disorder characterized by tall stature, intellectual disability and facial dysmorphism. This disorder is associated to a functional mutation in DNMT3A, an enzyme responsible for establishing DNA methylation implicated in gene regulation, and vital for development and cellular identity. Currently, we do not know how functional mutations in the DNMT3A protein can be at the origin of the neurodevelopmental and other associated problems observed in patients with TBRS. With the collaboration of clinical geneticists, we have identified 2 TBRS patients, carrying a single mutation in the functional methyltransferase domain of DNMT3A. Using cells from TBRS patients, we derived induced-pluripotent stem cells (iPSC), and reprogrammed these cells into neural progenitors and terminally differentiated neurons to establish the first preclinical model of TBRS to specify the deleterious impacts of functional DNMT3A mutations on brain cell development. We postulate that pathogenic heterozygous DNMT3A mutations lead to DNA methylation defects that will alter the normal programming of neural progenitors into differentiated neurons, interfering with neurodevelopmental events and ultimately leading to disease pathogenesis in TBRS. Our focused research aims are

1.Do pathogenic DNMT3A mutations alter cell lineage specification and differentiation processes of neural progenitors and terminally differentiated neurons?

2.Do pathogenic DNMT3A mutations alter the epigenetic program in neural progenitors and differentiated neurons?

This project will uncover the functional impact of DNMT3A mutations on the epigenome during brain cell development and provide a patient-derived model to test new therapeutic avenues to treat patients with TBRS.

Characterization of the Ser/Thr phosphatase PPEF1 in spermatozoa

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Sperm capacitation is a maturational process required for fertilization that involves protein phosphorylation and dephosphorylation signalling events. Calcium is a major actor in this process but the role and importance of Ca^{2+}-dependent calmodulin binding proteins in sperm capacitation remain to be established. Protein phosphatase with EF-hand domain 1 (PPEF1) was recently identified by our group after calmodulin affinity pull-down and mass spectrometry. PPEF1 is a Ser/Thr phosphatase that possesses an IQ-motif and several EF-hand domains. Our hypothesis is that PPEF1 could play a role in capacitation and in the control of hyperactivation in spermatozoa. The objectives of this study are to identify and localize PPEF1 isoforms by molecular approaches and to determine the contribution of this phosphatase in capacitation by enzymatic assays *in vitro*.

Four putative PPEF1 isoforms have been identified by RT-PCR of bovine testis RNA. Indirect immunofluorescence showed that PPEF1 was localized in the neck of uncapacitated spermatozoa in different species. PPEF1 is associated to sperm membranes, as confirmed by sperm fractionation and acyl-biotin exchange. Immunoprecipitation of PPEF1 allowed the identification of putative interactors and the measurement of phosphatase activity, but further studies are required to determine the effect of capacitation on PPEF1 localization and phosphatase activity.

This is the first study to establish the presence of PPEF1 in a reproductive biology and sperm function context. The broader objective of this project is to better understand the molecular mechanisms involved in sperm fertilizing ability.

Investigating Heritable Epigenetic Dysregulations in Promoter Regions Following a Temporary Inhibition of Dnmt1 in Mouse Embryonic Stem Cells

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In preimplantation mice embryos, a major reprogramming wave resets genome-wide DNA methylation (DNAmet) profiles. Differentially methylated regions (DMRs) (i.e., imprinted genes) must escape reprogramming and sustain precise DNAmet profiles through continuous DNMT1 (DNA methyltransferase 1) activity to ensure the proper establishment of the fetus's epigenome.

Using an embryonic stem (ES) cell model with inducible *Dnmt1* repression (*Dnmt1*$^{tet/tet}$), we showed that a temporary lack of *Dnmt1* triggers the permanent loss of DNAmet profiles on DMR and DMR-like regions. We still do not understand why these regions are unable to re-establish their DNAmet profiles following *Dnmt1* re-expression. Here we aim to define how a temporary lack of DNAmet maintenance remodels the chromatin landscape at genome-wide regulation regions such as promoters and how it modulates associated gene expression. *Dnmt1* expression in *Dnmt1*$^{tet/tet}$ ES cells was inhibited by adding doxycycline (2ug/mL) into the culture medium. Cells were collected prior to treatment, after a treatment of 6 days, as well as after 21 days of recovery. We performed RNA-Seq, Reduced-Representation Bisulfite Sequencing (RRBS) and Chromatin Immunoprecipitation (ChIP-Seq) for *H3K4me3*, *H3K4me1, H3K27me3* and *H3K27ac*. Our results showed that a subset of the 18,173 identified promoters that lost their methylation after Dox treatment, altering gene regulation. We'll soon start to analyse enhancers regions to identified if those regions are particularly impacted by the loss of DNA methylation maintenance and how it affects gene expression. Altogether, our analyses will shed light on the epigenetic mechanisms and impact on gene expression caused by a temporary loss of Dnmt1.

Characterization of micro-peritoneal metastasis in the mouse ovarian cancer model

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Introduction: High grade serous ovarian cancers (HGSCs), the most common ovarian malignancy, are rarely curable. This high mortality rate is due to late diagnosis in advanced stages with metastasis throughout the peritoneum, which leads to poor survival, while patients with localized HGSCs have higher survival rate. Therefore, it is essential to understand mechanism of HGSCs dissemination and how the peritoneum microenvironment interacts with micrometastases to develop novel therapeutic approaches that target this crucial step to improve long-term survival. **Methods:** To address these challenges, we develop a novel strategy to derive HGSCs in mice with CRISPR-mediated genome modification, Cre-induced fluorescence tracing and *in vivo* electroporation. This strategy possesses the capability of tracing clonal expansion and metastasis via fluorescence and the advantages of testing various mutation combinations in a limited region. After HGSCs in mice have metastasized, we extract different parts of the peritoneum and stain with various immune and extracellular markers to investigate interactions between mesenchymal microenvironment in peritoneum and HGSCs micrometastases. **Results:** We generated two mouse HGSC models by targeting three or four tumour suppressor genes, *TP53*, *BRCA*1 and *PTEN* with/without *LKB1*. Within these two mouse models, we observed various unique patterns of how fluorescent-labelled HGSCs micrometastases land onto the mesenchymal layer of peritoneum and recruitment of immune cells such as the $LYVE1^{+}$ macrophages towards the micrometastases.**Conclusions:** With this unique mouse model, we can gain more understanding of the key cellular interactions between HGSCs and the local peritoneal environment and the crucial events such as recruiting various immune cells during metastasis. My study can provide insights into the pathways critical for HGSCs metastasis which could serve as therapeutically targets.

Transcription of a novel inhibin B co-receptor is regulated by steroidogenic factor 1

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Inhibin A and B are gonadal hormones that suppress synthesis of follicle-stimulating hormone (FSH). They were previously thought to act through the same co-receptor, betaglycan. However, inhibin B action was maintained in gonadotrope-specific betaglycan knockout mice, suggesting that it might act via a distinct co-receptor. We recently discovered a novel inhibin B co-receptor, inhibin B binding protein (IBBP), that is selectively and highly expressed in gonadotropes. We also observed that *Ibbp* expression is essentially lost in mice with a gonadotrope-specific ablation of steroidogenic factor 1 (SF-1). Here, we describe our initial efforts to determine if and how SF-1 regulates *Ibbp* transcription.

We first performed 5' rapid amplification of cDNA ends to determine the transcription start site (TSS) of *Ibbp* and mapped two putative SF-1 binding sites 200 base pairs upstream of the TSS. We then ligated the proximal promoter to a luciferase reporter and compared promoter activity between heterologous HEK293T and homologous LβT2 gonadotrope-like cells. As expected, the basal promoter activity was higher in the homologous than heterologous cell lines. Co-expression of SF-1 with the promoter-reporter was sufficient to stimulate promoter activity in HEK293T cells. Mutating the two SF-1 binding sites decreased basal promoter activity in homologous cells and blunted the stimulatory effect of SF-1 co-expression in heterologous cells. Moreover, siRNA knockdown of SF-1 in the homologous cells decreased basal promoter activity. We are currently ascertaining whether SF-1 binds directly to the chromatin of the proximal promoter. Thus far, the data suggest that SF-1 contributes to gonadotrope-specific expression of *Ibbp*.

Mitochondrial network organisation in cumulus cells

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Mitochondria are key organelles that regulate a numerous cell processes such as ATP synthesis, steroid bioisynthesis, and apoptosis. In recent years, several studies have demonstrated that mitochondrial function is widely regulated by its structure. Within the cell, the morphology of this organelle varied between individual structures and complex network structures. In this study, we were interested in the characterisation of mitochondrial network organisation in porcine cumulus cells. Active mitochondria were stained with TMRM (Tetramethylrhodamine, Methyl Ester, Perchlorate) and observed with confocal microscopy. Images showed different mitochondrial network in cumulus cells. They were classified according to different categories (fragmented, intermediated, elongated and highly elongated). Thus, the network phenotype was defined by quantifying each cell depending on organelles structure. The results showed that most cells were having elongated mitochondrial phenotype. These results were validated by Momito algorithm demonstrating that mitochondrial length varied between 0.5 µm and 7 µm. Mitochondrial population was characterized with 25% of them with a length of about 1 µm and 40% of mitochondria were more than 2 µm in long. These observations were completed by Scaning Electron Microscopy (SEM) analysis showing that different mitochondrial structures ranged between rounded, small, intermediate and elongated forms. Our results support the presence of varied mitochondrial morphologies in cumulus cells. Since mitochondrial dynamic is a key regulator of mitochondrial function, the characterisation of mitochondrial network organisation allows us to further study the regulation of mitochondrial responses.

Early Detection of Abnormal Placental DNA Methylation and Gene Expression Associated with Assisted Reproduction: Differential Effects of Folic Acid Supplementation

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Assisted reproductive technologies (ART) have been associated with a higher incidence of adverse perinatal outcomes and epigenetic perturbations. Our group showed that ART is linked to increased embryonic developmental delay, a decrease in methylation at imprinted loci and global DNA methylation perturbations, the epigenetic defects being more pronounced in female placentas and partially corrected by moderate maternal folic acid supplementation. Our goal was to determine if the placental DNA methylation defects were accompanied by alterations in gene expression. RNA sequencing was used to examine the effect of ART in combination with folic acid supplementation in E10.5 female placentas and revealed that placentas from ART-conceived embryos exhibited 41 differentially expressed genes when compared to the control group. In contrast, Reduced Representation Bisulfite Sequencing identified a total of 2693 genes with DNA methylation perturbations. As for genome-wide DNA methylation, where folic acid supplementation corrected 8.7% and 7.1% of ART-induced perturbations (moderate and high dose, respectively), a trend toward correction of the differentially expressed genes was observed with the moderate dose of folic acid in the placenta. Amongst the differentially expressed genes in ART, we selected key genes involved in early trophoblast differentiation and angiogenesis to be examined closely at the level of DNA methylation and gene expression in the entire cohort. Together, the results suggest that DNA methylation perturbations early in gestation may precede effects on placental gene expression which may in turn contribute to the abnormal placentation observed later in gestation among mouse and human ART-conceived pregnancies. Funded by CIHR

Réorganisation des sites de liaisons de LRH1 suite au signal ovulatoire chez les cellules de la granulosa murine

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Le processus de la folliculogénèse est un phénomène ovarien méthodiquement orchestré afin de mener à bien l'ovulation et permettre le maintien de la gestation. Les cellules de la granulosa participent activement à ce processus. Elles nécessitent l'action de l'hormone folliculo-stimulante (FSH) qui leur permet d'entrer en de prolifération et l'action de l'hormone lutéinisante (LH) qui leur permet d'initier la phase de différentiation pour former le corps jaune. Le corps jaune assure le maintien de la gestation via la production de progestérone. Il a été démontré que LRH-1 (*liver receptor homolog-1*), un récepteur nucléaire exprimé exclusivement dans les cellules de la granulosa, joue un rôle crucial dans la fonction ovarienne pour la régulation d'un programme d'expression génique spécifique à chaque phase. L'étude d'un ChIP-seq de LRH-1 a montré qu'il subit un grand changement dans sa liaison à l'échelle du génome dans les cellules de granulosa suite à la LH. Notre hypothèse de recherche est que la LH induit un remodelage de la chromatine via le changement des sites de liaisons aux éléments de régulation distaux de LRH-1 permettant une réorganisation rapide du programme d'expression génique dans les cellules de la granulosa murine. L'étude des sites préférentiellement liés par LRH-1, variant dans des courts laps de temps, et quels en sont les répercussions sur les modifications épigénétiques seront mis en lumière grâce à la toute récente technique du Cut and Tag qui sera notre approche pour évaluer ces changements épigénétiques que nous combinerons avec les données des sites de liaisons de LRH-1.

Rôle immunomodulateur de TGFβ1 sur les voies inflammatoires induites par l'INFγ et le GM-CSF dans les trophoblastes et les macrophages humains

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Problématique. Selon leur profil inflammatoire (M1 ou M2), qui change en fonction de leur environnement, les macrophages utérins sont connus pour assurer autant le succès que l'échec de l'implantation embryonnaire et le développement fœtal. Ainsi, au cours de la gestation, une activation non régulée des voies pro-inflammatoires des macrophages M1, telles que INFγ/STAT1 et GMCSF/STAT5, peut entraîner une perte prématurée de l'embryon. Le rôle régulateur des cytokines gestationnelles comme le TGFβ1 sur l'activation de ces voies pro-inflammatoires dans les macrophages et les trophoblastes reste encore inconnu. **Hypothèse** : Le but de notre étude visait ainsi à déterminer si TGFβ1 inhibe la phosphorylation des facteurs STAT1 et STAT5 induits respectivement par l'IFNγ et le GM-CSF. **Méthodologie**. Les macrophages dérivés des cellules THP-1 et les cellules trophoblastiques JEG-3 ont été prétraitées pendant 3 heures avec 5 ng/mL d'IFNγ ou de GM-CSF. Les cellules ont ensuite été stimulées avec 50 ng/mL de TGFβ1 à différents périodes de temps (0 à 120 minutes). Des extraits protéiques ont été préparés et analysés pour la détection de phospho (p)-STAT1 et p-STAT5. **Résultats**. Nous avons observé que l'effet inhibiteur optimal de TGFβ1 sur STAT1 et STAT5 varie entre selon le type cellulaire et débute après 15 à 30 minutes de stimulation. Cet effet demeure stable dans les deux lignées cellulaires jusqu'à la 120ème minute de stimulation. **Conclusion**. Nous rapportons pour la première fois l'effet inhibiteur du TGFβ1 sur les voies proinflammatoires activées avec l'IFNγ et le GM-CSF dans les macrophages et les cellules trophoblastiques humaines.

Development of a highly sensitive enzyme-linked immunosorbent assay (ELISA) for measurement of follicle-stimulating hormone (FSH) in serum, plasma, and whole blood in mice

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Follicle-stimulating hormone (FSH) is produced and secreted by gonadotrope cells of the anterior pituitary gland. In females, FSH stimulates estrogen production and ovarian follicle development. In males, FSH promotes Sertoli cell proliferation, thereby regulating spermatogenesis. Circulating FSH levels are routinely measured in the diagnosis of reproductive dysfunction. Determination of FSH levels in serum and plasma were first performed by radioimmunoassay (RIA). Although still considered the gold standard, FSH RIAs require relatively large sample volumes, which can be limiting in smaller animals. Time-resolved immunofluorometric and enzyme-linked immunosorbent assays (ELISA) were developed to improve sensitivity and eliminate the need for radioactivity for FSH determination. These assays are often limited to serum or plasma and can be expensive when using commercial sources. Recently, several novel assays were developed to measure pituitary hormones like growth hormone, prolactin, and luteinizing hormone in mice from small sample volumes and at low cost. We developed and validated a novel and sensitive ELISA that enables the accurate measurement of FSH in serum, plasma, and whole blood from female and male mice. The assay can also be adapted to measure FSH in pituitary lysates and culture media from a gonadotrope-like cell line (LβT2 cells) or primary pituitary cultures. The results from this ELISA are highly correlated with those obtained with commercial assays. In summary, the new methodology described here will enable investigators to measure FSH from a variety of biological samples in mice accurately, at low cost, and in their own laboratories.

Cytokinesis fails to scale to cell size in the early mouse embryo

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Cytokinesis, the final step of cell division, is achieved by the assembly and constriction of an actomyosin ring that separates the daughter cells. Understanding cytokinesis regulation is key, as its failure leads to binucleation, which is a common feature of human embryos in IVF clinics and compromises embryo health. Cells halve in size with each division in early embryos, and how the actomyosin ring adapts to changing cell size is unknown. We therefore applied high resolution imaging and micromanipulation techniques to investigate the role of cell size in regulating constriction speed in mouse embryos. Our experiments demonstrate that constriction speed is independent of cell size, with similar speeds between embryos at the 1-cell (9.73±0.69µm/min), 2-cell (9.40±0.66µm/min), 4-cell (10.15±0.45µm/min) and 8-cell stages (9.65±0.63 µm/min). Similarly, embryos with artificially-reduced cytoplasm had constriction speeds (7.39±0.78µm/min) comparable to sham-manipulated embryos (7.22±0.41µm/min). Inhibition of actin polymerisation at mid-cytokinesis prevented cytokinesis completion, indicating that active turnover of F-actin is required for constriction. Interestingly, at the 16- and 32-cell stages, constriction speed was lower in outer cells (16-cell: 5.39±0.15µm/min; 32-cell: 4.71±0.67µm/min) than inner cells (16-cell: 7.71±0.42µm/min; 32-cell: 6.39±0.24µm/min). This reduction was not due to the emergence of cell-cell adhesion, as outer cells from embryos devoid of adhesion still displayed lower constriction speed (6.01±0.39µm/min) than inner cells (7.42±0.27µm/min). Our results indicate that constriction speed is independent of cell size in mouse embryos and instead, we propose that morphogenetic cues emerging at the 16-cell stage decrease constriction speed, potentially rendering outer cells to be more susceptible to cytokinesis failure.

Development of a new method for pig karyotyping

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Chromosomal rearrangements are widely recognized as a potential cause of subfertility in livestock production. In Canadian boar population, chromosomal rearrangement frequency has been estimated at 1.64%, and a chromosomal abnormality can be detected in approximately 50% of hypoprolific boars. Reciprocal translocations reduce litter size by 40% on average, leading to serious economic lost (4.6 million $ in Canada). In France, a mandatory systematic cytogenetic screening was implemented in the 90s to remove hypoprolific boars from artificial insemination centres. However, this screening program is not well developed in Canada and only a few resources are available for chromosome analysis. The Centre d'insémination porcine du Québec (CIPQ) wishes to provide such service but under current methods, obtaining accurate and repeatable results can be an issue since cytogenetic techniques require experienced users.

The aims of this study are to (1) establish a robust cost-effective method for chromosome labeling, (2) develop software for chromosome band patterns recognition, (3) build a graphical interface for data analysis, (4) train the staff at CIPQ to be able to use the tools.

To date, lymphocyte cell culture has been implemented and conventional chromosomal banding techniques have been tested. Many parameters influencing chromosome spreading and banding have been optimized.

Ultimately, this technological transfer project will develop a streamlined cytogenetic standard operation procedure that will be used at CIPQ to identify chromosomal abnormalities in boars and sows before being put in service.

This project is supported by a grant from the program Innov'Action from the Ministère de l'agriculture et des pêcheries du Québec.

Rôle régulateur de l'Oncostatine M (OSM) dans les trophoblastes en présence de signaux pro-inflammatoires

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Durant la grossesse l'activité immunitaire assure non seulement la protection et la défense de la mère et de son enfant mais joue également un rôle essentiel dans le support de celle-ci. En effet, la présence transitoire de facteurs pro-inflammatoires initie la mise en place de la gestation. Cependant leur activité doit être étroitement modulée par l'intervention de facteurs anti-inflammatoires au risque d'entraver le maintien de la grossesse. Ainsi pour limiter l'activité pro-inflammatoire et préserver l'intégrité de l'embryon et des tissus utérins, l'intervention de facteurs immunitaires gestationnels anti-inflammatoires comme l'Oncostatine M (OSM) est essentielle. Principalement exprimé au niveau intra-utérin chez la femme enceinte notamment lors de l'implantation embryonnaire et participant à la régulation des signaux pro-inflammatoire durant la phase-aigue de l'inflammation, l'OSM pourrait également être impliqué dans la modulation de l'inflammation à l'interface foeto-maternel au cours de la gestation. Cependant ses mécanismes d'actions restent méconnus.

Ainsi nos études démontrent l'effet modulateur de OSM dans la réponse des trophoblastes humains (BeWo) traités avec des milieux conditionnés de macrophages M1 (M.C M1) (THP-1) ou en présence des facteurs pro-inflammatoires IFN□ et GM-CSF. Nous mettons également en évidence certains mécanismes intra-cellulaires et moléculaires pouvant permettre à l'OSM de moduler l'activation de voies pro-inflammatoires telles que p-STAT1 et p-STAT5 dans les Bewo.

Les convulsions fébriles sont associées à des altérations de l'expression de gènes dans le placenta à terme

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Les convulsions fébriles (CF) sont des convulsions en cas de fièvre chez le jeune enfant. Cette affection est commune, mais peu étudiée. Si des facteurs génétiques et immunitaires y sont associés, aucune étude ne s'est intéressée à la fonction placentaire. L'objectif de cette étude est de comparer chez des enfants ayant ou non fait des CF l'expression placentaire de gènes impliqués dans les systèmes sérotonine, glucocorticoïdes, stéroïdes et de croissance et développement. L'étude a été approuvée par le comité d'éthique du CHU Sainte-Justine. Les participantes ont été recrutées au sein de l'étude cohorte 3D (CF, n=30 ; témoin, n=33). L'expression placentaire des gènes impliqués dans les systèmes sérotonine (*TPH2, SLC6A4, MAO-A, HTR2A, HTR2*B), glucocorticoïde (*CRH, HSD11B1, NR3C-α, HSD11B2, NR3C-β*), stéroïde (*GEPR1, mPR, CYP19A1*) et croissance et développement (*GJA1, TPJ1, CSH1, KRT7, VEGFA*) a été mesurée par RT-qPCR. Les données ont été analysées par Anova suivie d'une correction fdr. Les enfants avec CF naissent plus légers que ceux du groupe témoin (p=0.03), sans différence de mensurations placentaires. À terme, l'expression de *SLC6A4* (p=0,03) et *TJP1* (p<0,01), est plus élevée dans le groupe CF. *GJA1* (p=0,06), *NR3C-α* (p=0,07), *CYP19A1* (p=0,07) et *GEPR1* (p=0,07) ont tendance à être différemment exprimés entre les groupes. La surexpression de gènes impliqués dans le développement placentaire ainsi que dans le système sérotonine indiquent une altération de la fonction placentaire chez les enfants CF. Les CF auraient ainsi une origine fœtale. D'autres analyses permettront d'en savoir plus sur le rôle de la fonction placentaire dans les CF.

XIST DMR methylation levels and knockdown in bovine somatic cells and SCNT-derived embryos

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XIST (X-inactive specific transcript) is a long non-coding RNA responsible for the random inactivation of one X chromosome during the development of the female conceptus in placental mammal. Apart from XIST expression, other epigenetic mechanisms are involved such as DNA methylation and histone modification. Abnormal X inactivation has been described with assisted reproductive techniques as somatic cell nuclear transfer (SCNT) using both female and male cells. Albeit well characterized in mice, little is known about XIST function in large domestic animals produced *in vitro* (IVF) and by SCNT. Thus, the overall goal of this work is **to investigate the role of XIST during X chromosome inactivation (XCI) in somatic cells and embryos derived by IVF and SCNT**. Specifically, we utilized small interference RNA (siRNA) to investigate the consequence of XIST knockdown in SCNT embryo production and the role of DNA methylation. Preliminary results show that the XIST DMR in sperm, oocytes, and both morula and blastocyst stage embryos is hypomethylated, while the female and male fibroblasts showed around 50% and 92% of methylation, respectively. Moreover, siRNA XIST downregulating (60%) in female fibroblasts did no alter blastocyst development, indicating that XIST downregulation in the donor cells does not affect development after SCNT. Further studies are planned to examine the effects of XIST knockdown on its DMR and the patterns of H3K27me3 using male fibroblasts and IVF embryos. Financial support from NSERC Canada (LS) and a postdoctoral fellowship from Mitacs/Boviteq (RS).

ZEB1 inhibits *Lhb* transcription by blocking the stimulatory actions of EGR1

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Luteinizing hormone (LH), a pituitary heterodimeric glycoprotein, regulates gonadal function in both males and females. LH is composed of an α subunit and a hormone-specific β subunit (LHβ, product of the *Lhb* gene). Hypothalamic gonadotropin-releasing hormone (GnRH) stimulates *Lhb* transcription by inducing the expression of early growth response 1 (EGR1). EGR1 then partners with steroidogenic factor 1 (SF1) and *paired*-like homeodomain transcription factor 1 (PITX1), which bind composite *cis*-elements in the *Lhb* proximal promoter. Female mice lacking microRNAs 200b and 429 are anovulatory and infertile due to a selective LH deficiency. In the absence of these miRNAs, protein expression of one of their targets, zinc finger E-box binding homeobox 1 (ZEB1), is increased in the pituitary. siRNA-mediated knockdown of ZEB1 in murine gonadotrope-like LβT2 cells increases *Lhb* mRNA levels. These data suggest that ZEB1 represses *Lhb* expression, but the underlying mechanisms were not fully elucidated. Here, we observed that ZEB1 over-expression blocked GnRH-stimulated murine *Lhb* promoter-reporter activity in LβT2 cells. The stimulatory effects of GnRH can be recapitulated by EGR1 over-expression. ZEB1 also inhibited EGR1 induction of *Lhb* promoter activity in LβT2 cells and in heterologous HEK293 cells. These data suggest that ZEB1 represses *Lhb* transcription by inhibiting the stimulatory actions of EGR1. We are currently determining whether and where ZEB1 binds the *Lhb* promoter. We are also investigating the functional domains in ZEB1 that mediate its repressive actions on *Lhb* expression.

Gonad-Specific Knockout of Steroidogenic Factor 1 (SF-1) leads to impaired fertility in male mice

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Steroidogenic factor 1 (SF-1) is an orphan nuclear receptor that plays an essential role in the development of fetal gonads and regulates the expression of genes involved in steroid biosynthesis. Since SF-1 is expressed in multiple cell types of the mouse testis, we developed three novel conditional KO (cKO) mouse models to identify the role of SF1 in the male gonad; Cytochrome P450 17α-hydroxylase (Cyp17-Cre/SF-1, Leydig cell specific) Cre-recombinase, aromatase (Cyp19-Cre/SF-1) Cre-recombinase, as well as a combination of these two (Cyp17-Cyp19-Cre;SF-1). When compared to control animals, Cyp19-Cre/SF-1 cKO males showed normal fertility and testicular function. The Cyp17-Cre/SF-1 cKO males had smaller testis, with drastically reduced Leydig cell volumes and impaired steroidogenesis, though their reproductive performance remained comparable to controls. 50% of Cyp17-Cyp19-Cre/SF-1 double-cKO males were infertile, while the other 50% showed significantly reduced fertility. Double cKO males also had smaller testis with degenerative seminiferous tubules and reduced Leydig cell population. While the expression of most steroidogenic genes was significantly reduced in the double-cKO testis, FSH receptor and aromatase transcript abundance were increased, indicating an abnormally elevated production of 17b-estradiol. This effect, linked to a reduced level of testosterone, could explain the loss of fertility in these males. This significant difference in fertility between our three models demonstrates the essential role of SF-1 in male testicular function, that the presence of SF-1 in one cell type is able to compensate for the absence in another, and that the depletion SF-1 in all steroidogenic cells of the testis leads to impaired fertility.

Gene cascades analysis following human granulosa tumor cell (KGN) exposure to high levels of free fatty acids and insulin

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During metabolic stress such as obesity or diabetes, adipose tissue is more likely to release free fatty acids (FFA) in serum resulting in an increase of FFA levels not only in blood but also in follicular fluid (FF). In humans, high concentration of palmitic acid (PA) and stearic acid (SA) were shown to reduce granulosa cells survival and higher follicular FFAs were associated with poor COC morphology. Obesity and high levels of circulating FFAs have also been causatively linked with hampered insulin sensitivity in cells and compensatory hyperinsulinemia. To provide a global picture of the principal upstream signaling pathways and genomic mechanisms involved in this metabolic context, human granulosa-like tumor cells (KGN) were treated with combinations of PA, oleic acid (OA), SA and insulin at high physiological concentrations found in women follicular fluid with higher BMI (BMI ≥ 30.0 kg/m^2). Results from RNA-seq analysis showed thousands of differentially expressed genes for each treatment with cut off for fold change of 1.5 and p-value 0.05. Using analysis software such as Ingenuity pathway analysis (IPA), we were able to establish that high concentrations of FFA affected the expression of genes mainly related to glucose and insulin homoeostasis, fatty acid metabolism as well as steroidogenesis and granulosa cells differentiation processes. The combination of insulin and high concentration of FFAs showed signalling pathways related to apoptosis, inflammation and oxidative stress. Taken together, our results provide new information on the mechanisms that might be involved in human granulosa cells when exposed to high concentrations of FFA and insulin in a context of metabolic disorders.

The Forensic Science behind the Caribou (*Rangifer*) SNP Chip Validation

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The Ministère des Forêts, de la Faune et des Parcs du Québec (MFFP) develops and implements tools to better monitor, manage and protect wildlife species. Genomic has recently been added to this toolbox through a genotyping SNP chip. In addition to monitoring genetic evolution by metrics, such as population structure and inbreeding levels, a genotyping chip will also serve as a wildlife forensic tool by determining the population of origin for anti-poaching law enforcement.

Our aim was to document the specifications of the new caribou (*Rangifer*) SNP chip by addressing its robustness, sensitivity, repeatability, reproducibility, specificity and ability to distinguish mixed samples. We first focused on sample preparation. DNA was extracted from hair follicles, muscle biopsies, ear punches, and fecal pellets. Samples generated different DNA quality and quantity outputs. Various extraction methods have been tested to obtain high molecular weight DNA and to remove PCR inhibitors. A qPCR assay was designed for sexing purposes and to estimate the quantity of caribou DNA from fecal pellets.

The SNP chip design and manufacturing are underway. Specificity will be tested using DNA samples from other wild ungulates. Impact of DNA quantity and integrity will be tested by hybridizing different samples varying in their DNA fragmentation degree. Population assignment power will be determined using a user-blind assay where genotypes will be used to calculate LOD scores (logarithm of the odds) to infer population of provenance. These validation steps are crucial to develop a powerful tool that will fulfill various uses, especially in wildlife forensic.

Effects of organophosphate ester flame retardants on KGN cells, a human granulosa cell line

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Organophosphate ester (OPE) flame retardants are now used extensively to replace polybrominated diphenyl ethers (PBDEs) and are found ubiquitously in the environment. However, there is little information available on the safety of OPEs. Previous studies suggest that exposure to OPEs may be detrimental to female fertility. To test the hypothesis that OPEs alter the function of ovarian granulosa cells to a greater extent than PBDEs, we compared the effects of a major PBDE, 2,2',4,4' tetrabromodiphenyl ether (BDE-47), to those of five commonly used OPEs, tris(methylphenyl) phosphate (TMPP), triphenyl phosphate (TPHP), isopropylated triphenyl phosphate (IPPP), *tert*-butylphenyl diphenyl phosphate (BPDP), and tributoxyethyl phosphate (TBEP). KGN immortalized human granulosa cells were exposed to BDE-47 or an OPE (0.001 – 100 µM) for 48h. Effects on cell counts, lysosomes, lipid droplets, and oxidative stress were determined using fluorescent dyes and high-content imaging. Benchmark concentration (BMC) analyses were done to estimate the concentrations that induce a 10% change from control. The cytotoxicity of most OPEs was comparable to BDE-47. TMPP, IPPP, and BDE-47 decreased the numbers of lysosomes in cells. All of the chemicals tested increased the total area of lipid droplets in cells, with BMC values for TMPP < IPPP < BPDP = TPHP < BDE-47 < TBEP. Only TMPP and IPPP induced oxidative stress. In summary, most of the OPEs have BMC values lower than that of BDE-47 for at least one of the phenotypic endpoints tested, suggesting these alternatives may be more toxic than the PBDEs they have replaced. Supported by CIHR and McGill University.

Functional effects of Tribbles Homolog 2 (TRIB2) in granulosa cells of ovarian follicles

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Tribbles homolog 2 (TRIB2) is a member of the serine/threonine kinase superfamily. We previously reported that TRIB2 is differentially expressed in granulosa cells (GC) of bovine preovulatory follicles. This study aimed to investigate TRIB2 function and its binding partners in GC of bovine ovarian follicles.

GC were obtained from bovine follicles at different stages of development: small follicles (SF), dominant follicles (DF) at day 5 of the estrus cycle and ovulatory follicles (OF) at 0, 6, 12, 18 and 24 hours post-hCG injection. An *in vitro* model of cultured GC was used for functional studies using the CRISPR-Cas9 approach and the pQE system, respectively to inhibit and overexpress TRIB2. The yeast two-hybrid approach was used to identify TRIB2 binding partners.

RT-qPCR analyzes showed greatest expression of *TRIB2* mRNA in GC of DF as compared to OF ($P<0.0001$) and a significant decrease in *TRIB2* mRNA expression from 6h up to 24h post-hCG as compared to 0h ($P<0.001$). *In vitro*, TRIB2 inhibition resulted in increased GC proliferation and *CYP19A1* expression ($P<0.05$). Western blot analyses showed reduction in ERK1/2 (MAPK3/1) and p38MAPK (MAPK14) phosphorylation levels following TRIB2 inhibition, while TRIB2 overexpression increased p-ERK1/2 and p-p38MAPK ($P<0.05$). Yeast two-hybrid screening revealed INPPL1, CALM1, RAB14, SCD, SDHB, NT5E and INHBA as TRIB2 binding partners. Further analyses showed that TRIB2 inhibition/overexpression led to significant changes in binding partners expression.

These results demonstrate that TRIB2 modulates MAPK and target partner signaling in GC affecting GC proliferation, steroidogenesis, and final follicle development.

Structure and assembly dynamics of the kinetochore in oocyte meiosis-I

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Oocyte meiosis-I is a highly idiosyncratic cell division in which chromosomes are frequently missegregated leading to aneuploidy. The central player in chromosome segregation is kinetochore, which binds to both chromosomal DNA and spindle microtubule to mediate chromosome alignment and segregation. A detailed understanding of kinetochore structure and function in mammalian oocytes is lacking. Immunofluoescence revealed stable localization of CENP-C and CENP-T throughout meiosis-I, declining upon meiosis-I completion. Comparing to the other components, Ndc80 wasn't initially present at GV-stage centromeres, but became rapidly recruited upon resumption of meiosis-I. To examine the role of major M-phase kinases in kinetochore assembly we used chemical inhibitiors of CDK-1 (Roscovitine), and AurK (Hesperadin, ZM447439). Inhibition of AurK activity before meiosis resumption could significantly diminish Ndc80 assembly, suggesting a key role for AurK activity in kinetochore assembly in oocytes. In contrast to somatic cells, kinetochore assembly appears independent of CDK1 activity in mouse oocytes. Suppression of either CDK1 or AurK pathways after GVBD was insufficient to disassemble kinetochore. Using centroid fitting to establish relative positions of kinetochore subunits, we find that the kinetochore is under mechanical tension in late meiosis-I, leading to intra-kinetochore stretch between specific subunits. A better understanding of kinetochore structure and function will be essential in unravelling the causes of chromosome segregation error that leads to oocyte aneuploidy and age-related infertility.

The role of Janus Kinase 3 (JAK3) in bovine ovarian granulosa cells.

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Janus kinase 3 (JAK3) is a tyrosine kinase protein, which functions through the JAK/STAT pathway. *JAK3* is differentially expressed in granulosa cells (GC) of bovine preovulatory follicles and is downregulated in ovulatory follicles by the endogenous luteinizing hormone (LH) and hCG injection. Based on these observations, JAK3 signaling could modulate GC proliferation and follicular growth. Our objective was to analyze the effects of JAK3 inhibition/overexpression in the phosphorylation of target proteins including STAT proteins and newly identified JAK3 binding partners CDKN1B and MAPK8IP3. First, GC were obtained from small follicles (SF), dominant follicles (DF), ovulatory follicles (OF) in order to analyze *in vivo* regulation of JAK family members. Second, GC from slaughterhouse ovaries were cultured and treated with or without FSH and Janex-1, a JAK3 inhibitor, and samples were analyzed by RT-qPCR and western blotting. RT-qPCR analyses using *in vivo* samples showed that JAK members were differently regulated in different follicle stages and CL. *In vitro* experiments revealed that Janex-1 treatment significantly decreased JAK3 expression in GC while FSH tended to increase JAK3 expression. Furthermore, steady-state mRNA expression for steroidogenic enzymes *CYP19A1* and *CYP11A1* and proliferation markers *PCNA* and CCND2 were upregulated in GC with FSH treatment and significantly decreased with Janex-1 treatment as compared to control. Western blot analysis showed that JAK3 overexpression increased STAT3 phosphorylation while Janex-1 treatment reduced STAT3 phosphorylation levels. However, FSH treatment partially rescued STAT3 phosphorylation in Janex-1-treated cells. These results suggest that JAK3 plays a key role in GC proliferation, follicular growth and steroidogenesis.This work was supported by a Discovery grant from the National Sciences and Engineering Research Council of Canada (RGPIN#04516 to KN).

Ovariectomy-induced bone loss may be prevented in FSH-deficient mice

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Postmenopausal osteoporosis has been attributed to decreased estradiol levels. In the hypothalamic-pituitary-gonadal (HPG) axis, estradiol synthesis is stimulated by follicle-stimulating hormone (FSH). FSH is secreted from the anterior pituitary gland and estradiol feeds back to the hypothalamus and pituitary to suppress FSH production. In postmenopausal women, the loss of estradiol negative feedback leads to elevated FSH levels. It was hypothesized that this increase in FSH also contributes to postmenopausal bone loss. If FSH indeed has noncanonical actions in bone, antagonism of FSH might represent an effective preventative measure or treatment for postmenopausal osteoporosis. Therefore, our objective was to test whether FSH suppression ameliorates ovariectomy-induced bone loss in mice. We used an inducible conditional knockout of *Foxl2* and *Smad4* (*Foxl2*$^{fx/fx}$;*Smad4*$^{fx/fx}$;iGRIC/+), transcription factors important for FSH production, as a model to suppress circulating FSH levels in adult mice. Ten-week-old female mice were subjected to ovariectomy or sham operation and were injected with tamoxifen (2 mg/injection) to induce the recombination of *Foxl2* and *Smad4*. Mice were sacrificed at 19-weeks of age for skeletal analysis. Ovariectomy induced a nonsignificant decrease in femoral bone mineral density (BMD) of control (*Foxl2*$^{fx/fx}$;*Smad4*$^{fx/fx}$), but not in FSH-deficient mice (*Foxl2*$^{fx/fx}$;*Smad4*$^{fx/fx}$;iGRIC/+). Animals with reduced FSH levels had higher BMD compared to controls, independent of surgery type. In conclusion, ovariectomy-induced bone loss may be blocked in mice with reduced FSH levels. We are currently investigating the mechanisms of FSH action on bone using additional genetically-modified mouse models.

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"Adult marmoset fallopian tube-uterus junction"